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TITLE

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

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KEY WORDS

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ABSTRACT

Objective: To clarity the effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine, and to create standards to adjust the doses of these drugs in clinical practice based on the smoking status.

Design: A meta-analysis was conducted by searching MEDLINE for relevant prospective and retrospective studies.

Included Studies: We included the studies that investigated the effects of smoking on the concentration to dose (C/D) ratio of olanzapine or clozapine.

Primary outcome measure: The weighted mean difference was calculated using a DerSimonian-Laird random effects model. Heterogeneity was assessed by the χ^2 test and quantified by I^2.

Results: Seven association studies of olanzapine were included in the meta-analysis of olanzapine. The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p< 0.00001$) and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Four association studies of clozapine were included in the

meta-analysis of clozapine. The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratios was significantly lower in smokers than in non-smokers ($p < 0.00001$) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70).

Conclusions: This meta-analysis synthesized previous studies and determined the impact of smoking on the disposition of olanzapine and clozapine in a way that can be used to change clinical practices. These results are useful as standards to adjust the doses of olanzapine and clozapine based on the smoking status in clinical practice.

270 words

ARTICLE SUMMARY

Article focus

- Many studies related to the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but there has been no definitive agreement regarding the dose adjustment needed in clinical practice based on the smoking status.
- The meta-analyses of prospective and retrospective studies were conducted to clarify the effects of smoking on the disposition of olanzapine and clozapine and to create standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

Key messages

- The mean difference in the concentration to dose (C/D) ratios of olanzapine between smokers and non-smokers was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). It was estimated that when 10 mg/day of olanzapine (the usual dose in Japan) would be administered to non-smokers, about 13 mg/day should be administered to smokers in order to obtain the equivalent olanzapine concentration

as in non-smokers.

- The mean difference in the C/D ratios of clozapine between smokers and non-smokers was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). It was estimated that when 200 mg/day of clozapine (the usual dose in Japan) would be administered to non-smokers, about 360 mg/day should be administered to smokers, in order to obtain an equivalent clozapine concentration.

Strengths and limitations of this study

- The major strength of this study is that it clarifies the effects of smoking on the olanzapine and clozapine concentrations in a large population and provides standards that can be used to regulate the dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status.
- The major limitation of this study is the paucity of studies included. This meta-analysis standardized pharmacokinetic parameters to C/D ratios, and therefore, only seven studies for olanzapine and four studies for clozapine could be included.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug approved for the treatment of schizophrenia, mania and for preventing the recurrence of bipolar disorders[1]. Olanzapine is a thienobenzodiazepine derivate, which shows potent antagonism at $D_{1\text{-}4}$ dopaminergic receptors, as well as 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[2]. Olanzapine is extensively metabolized in the liver, mainly via cytochrome P450 (CYP) 1A2, but also via CYP2D6, CYP3A4, flavin-containing monooxygenase and via glucuronidation[2]. Among these enzymes, CYP1A2 accounts for approximately 50% to 60% of olanzapine metabolism[2].

Clozapine is the prototype atypical antipsychotic, and it belongs to the chemical class of the dibenzodiazepines[1]. Clozapine has much greater antagonistic activity on D_4 than D_2 dopaminergic receptors. It also showsa potent antagonism at 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[1]. Clozapine has been widely used following its introduction, because it induces relatively few extrapyramidal effects, and it shows therapeutic benefits for patients who have failed to respond to other agents[3]. Clozapine is rapidly absorbed, and undergoes extensive hepatic metabolism[4]. Various lines of evidence indicate that CYP1A2 and CYP3A4 play a significant role in both *N*-oxidation and *N*-demethylation of the

compound, whereas CYP2D6 plays a minor role in *N*-demethylation[1 4].

The prevalence of smoking is two- to three-fold higher in patients with schizophrenia than that in the general population, and about 58-88% of patients with schizophrenia are current smokers[5]. Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of many drugs, including olanzapine and clozapine[6].

Previous clinical studies reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total (BPRS) score than non-smokers[7 8]. It was also reported that smokers who were treated with clozapine suffered side effects (i.e. auditory hallucinations, hallucinations, hypersalivation, drowsiness, clonic seizures, convulsions and unconsciousness) after smoking cessation[4 9-12].

Many studies about the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but no definitive agreement regarding the dose adjustment in clinical practice based on the patient's smoking status has been reached. There are several reasons for the slow progress in making the smoking-associated dosage selection; (i) the sample sizes of the previous studies were small; (ii) each study used different pharmacokinetic (PK) parameters [e.g., plasma concentration, plasma

concentration to dose (C/D) ratio, clearance (CL)] and the degree of the effect of smoking on the dispositions of olanzapine or clozapine was different between studies. Therefore, a meta-analysis has been needed to overcome the limitations of the previous studies and to determine the degree of the effects of smoking on the disposition of olanzapine and clozapine, in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on the smoking status of the patient.

In this study, we performed a meta-analysis of the effects of smoking on the disposition of olanzapine and clozapine.

METHODS

Study selection

A preliminary search of the literature covering the period from 1946 to August 2012 was undertaken to identify publications related to the effects of smoking on the disposition of olanzapine and clozapine. The electronic database, MEDLINE, was initially searched using six terms, in which either ‘olanzapine’ or ‘clozapine’ was paired with ‘smoking’ or ‘cigarette’ or ‘tobacco’ or ‘smoke’. We excluded other than English publications, and studies not performed on human participants, after the search. The

inclusion criteria were as follows: (i) published in a peer-reviewed journal; (ii) contained the mean C/D ratios (ng/mL)/(mg/day) of olanzapine or clozapine, and their standard deviation (SD) in smokers and non-smokers, respectively, and we requested data from the author(s) if the either the mean C/D ratios or the SD was not described; and (iii) the data were from subjects who had received olanzapine or clozapine for at least a week. Additionally, we divided the selected publications into two groups, i.e. olanzapine and clozapine study groups (Figure 1).

Data extraction

The number of patients, the mean values of the C/D ratios and the SD values of these ratios were extracted for smokers and non-smokers, respectively, from the selected publications. The C/D ratios were standardized to be in the same units, i.e. (ng/mL)/(mg/day). When the values were drawn on other scale [e.g., (ng/mL)/(mg/kg)], we asked the author(s) to send us their data in the desired units.

Statistical analysis

A meta-analysis using the weighted mean difference in the C/D ratios of olanzapine or clozapine between smokers and non-smokers was performed using the

Review Manager (RevMan) Version 5.1 for Windows software program (Cochrane Collaboration, http://www.cc-ims.net/RevMan). Cochran's chi-square-based Q-statistic test was applied to assess the between-study heterogeneity. The weighted mean difference was calculated using DerSimonian-Laird random effects models[13], along with 95% confidence intervals (CI), to measure the strength of the association. In this study, we applied the random effects model for the comparisons, which is more conservative because of the possibility that the underlying effect differed across studies and populations. We used the I^2 statistic to assess the heterogeneity of the results. Publication bias was assessed by visually examining a funnel plot with asymmetry and formally assessing publication bias with the Egger test[14]. The statistical significance level for all analyses was set at a two-sided value of $p<0.05$.

RESULTS

Olanzapine: Search results and study characteristics

Seven studies of olanzapine[15-21] met our criteria (Figure 1). The studies included in this analysis for olanzapine are listed in Table 1. Since the study by Citrome *et al.*, 2009[18] was derived from a randomized clinical trial of 10, 20, and 40 mg as the daily olanzapine dose in patients with schizophrenia or schizoaffective disorder, we divided

its populations into three groups according to the respective olanzapine doses. Since the study by Spina *et al*., 2009[19] focused on the effects of valproate on the olanzapine plasma concentrations, so we extracted the C/D ratios of olanzapine at baseline (before taking valproate). The study by Haslemo *et al*., 2011[21] focused on the effects of contraceptives on the serum concentration of olanzapine among female patients who were treated either with olanzapine alone or the combination of estradiol-containing contraceptives, so we requested the C/D ratios in subjects not using any contraceptives that can affect the CYP1A2 activity.

Table 1. The list of olanzapine studies

Study	Country	Study Design	N (smoker)	Gender (male/female)	Diagnosis	Age (mean ± SD)
Nozawa M *et al*., 2008	Japan	Retrospective study	51 (16)	34/17	Schizophrenia	32.6±9.6
Bigos KL *et al*., 2008	USA	Prospective study	406 (267)	289/117	Schizophrenia	42±7.9
Laika B *et al*., 2009	Germany	Retrospective study	73 (30)	36/37	Schizophrenia, Mood disorder	41.7±14.7
Citrome L *et al*., 2009	USA	Prospective study	380 (257)	265/115	Schizophrenia, Schizoaffective disorder	18 - 60

Spina E *et al*., 2009	Italy	Prospective study	18 (8)	10/8	Bipolar disorder, Schizoaffective disorder	39.3±8.6
Skogh E *et al*., 2011	Sweden	Retrospective study	37 (10)	25/12	Schizophrenia, Schizoaffective disorder	23 – 50
Haslemo T *et al*., 2011	Norway	Retrospective study	129 (64)	0/129	Unknown	18 – 40

Primary analyses of olanzapine

There was no significant heterogeneity among the mean differences (I^2=11%, p= 0.35) (Figure 2). The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 2), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI: -0.89 to -0.61). No significant bias was shown using the Egger test in the studies of olanzapine (p=0.282). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 1). Since we could not obtain the data regarding the olanzapine disposition during the condition of smoking cessation, the difference in the C/D ratios after smoking cessation could not be determined.

Subgroup analyses of olanzapine

Prospective studies

We conducted subgroup analyses to confirm the precision of the primary analyses. Of the seven included studies of olanzapine, three were prospective studies, while four were retrospective studies. In the prospective studies (532 smokers and 272 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3), and the mean difference was -0.73 (ng/mL)/(mg/day) (95% CI: -0.95 to -0.50).

Retrospective studies

In the retrospective studies (120 smokers and 170 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 4), and the mean difference was -0.84 (ng/mL)/(mg/day) (95% CI: -1.08 to -0.59).

Clozapine: Search results and study characteristics

Four studies regarding the clozapine disposition[22-25] met our criteria (Figure 1). The clozapine studies included in this analysis are listed in Table 2.

Table 2. The list of clozapine studies

Study	Country	Study Design	N (smokers)	Gender (male/female)	Diagnosis	Age (mean ± SD)
Dettling M *et al*., 2000	Germany	Retrospective study	34 (25)	18/16	Schizophrenia, Bipolar disorder	33.7±10.6
Palego L *et al*., 2002	USA	Retrospective study	49 (22)	25/24	Schizophrenia, Schizoaffective disorder	36.84±1.96 (SE)
Weide J *et al*., 2003	Netherlands	Retrospective study	80 (45)	51/29	Schizophrenia	18 - 86
Haslemo T *et al*., 2006	Norway	Retrospective study	33 (28)	21/12	Schizophrenia	52±9

Analyses of clozapine

There was no significant heterogeneity among the mean differences (I^2=33%, p=0.22) (Figure 5). The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 5), and the mean difference was -1.11

(ng/mL)/(mg/day) (95% CI -1.53 to -0.70). No significant bias was shown using the Egger test for the clozapine studies (p=0.436). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 2). In the meta-analyses of clozapine, no subgroup analyses could be conducted because of the small number of patients included in the study. We were not able to conduct a meta-analysis related to the effects of smoking cessation on the clozapine C/D ratios due to the small number of the patients involved.

DISCUSSION

Smoking is a well-known cause of significant drug interactions in humans[26-28]. The polyaromatic hydrocarbons in cigarette smoke are known to induce CYP1A2[29], and therefore, cigarette smoking can affect the disposition of drugs that are metabolized by CYP1A2, such as olanzapine and clozapine. The prevalence of current smokers is higher in patients with schizophrenia than that in the general population[5]. However, at present, there is no definitive data regarding the dose adjustments of olanzapine and clozapine in clinical practice based on the patient's smoking status. This is the first meta-analysis to clarify the effects of smoking on the disposition of these drugs.

Olanzapine

In the meta-analysis of olanzapine, 1094 patients (652 smokers and 442 non-smokers) from seven clinical studies of olanzapine were evaluated. The results showed that the C/D ratio of olanzapine was 0.75 (ng/mL)/(mg/day) lower in smokers than in non-smokers. The subgroup analyses (prospective/retrospective studies) also showed similar results. Approximately 85% of the oral olanzapine dose is absorbed, but as about 40% is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60%[1]. The mean half-life, mean apparent drug plasma CL and mean apparent volume of distribution of olanzapine were 33 hours, 26 L/h and 1150 L in healthy individuals[30]. Previous clinical studies demonstrated that the C/D ratio of olanzapine significantly correlated with a decrease in the BPRS[7 8]. The correlation between the clinical outcome and the plasma olanzapine concentration is clearly curvilinear, with clinical efficacy being approximately associated with a plasma olanzapine concentration range of 20-50 ng/mL[1]. Based on the findings of the present study, it was estimated that when 10 mg/day of olanzapine (the usual dose in Japan) would be administered to non-smokers, about 13 mg/day should be administered to smokers in order to obtain the equivalent olanzapine concentration.

Clozapine

In the meta-analysis of clozapine, 196 patients (smokers: 120, non-smokers: 76) from four clinical studies were evaluated. The results showed that the C/D ratio of clozapine was 1.11 (ng/mL)/(mg/day) lower in smokers than in non-smokers. After oral administration of clozapine, the drug is rapidly absorbed. Only 27-50% of the dose reaches the systemic circulation unchanged, because of extensive first-pass metabolism [1]. There is a wide inter-patitent variability in PK parameters of clozapine[1]. The mean half-life of clozapine ranges from 9 to 17 hours[1]. The plasma CL of clozapine was reported to be between 9 and 53 L/hour, and the volume of distribution of clozapine was between 2 and 7 L/kg[1]. The steady-state plasma concentrations of clozapine are reached after 7-10 days of dosing[1]. The relationship between the clozapine concentration and clinical outcome is controversial. According to the study by Spina *et al*., 2000[31], a receiver operating characteristics analysis showed that a clozapine concentration cut-off value of 350 ng/mL distinguished responders and non-responders with a sensitivity of 72% and a specificity of 70%. On the other hand, it has been suggested that the clozapine concentration does not correlate with the decrease in the BPRS[32 33].

A recent review summarized the previous studies regarding the relationships between the clozapine concentrations and clinical response, and suggested that

clozapine levels above 250-400 ng/mL are associated with an increased chance of a clinical response [34]. Moreover, clozapine doses exceeding 500-600 mg/day of clozapine could carry an increased risk of seizures[34]. Because the smokers who were treated with clozapine were reported to suffer serious central nervous side effects after smoking cessation[4 9-12], it is necessary to regulate the clozapine dosage carefully when smokers stop smoking or decrease the amount of smoking. Based on the findings of the present study, it was estimated that when 200 mg/day of clozapine (the usual dose in Japan) would be administered to non-smokers, about 360 mg/day should be administered to smokers in order to obtain an equivalent clozapine concentration.

Other factors affecting the disposition of olanzapine and clozapine

The pharmacokinetics of olanzapine and clozapine are affected by not only smoking, but also many other patient-related factors (e.g. sex, race, body weight, genotype). A previous clinical study reported that sex, race and the *CYP1A2* genotype could affect the CL of olanzapine [16], whereas another study reported that the sex, age and weight could affect the plasma concentration of clozapine[35]. This meta-analysis simply analyzed the effects of smoking, and did not take these other factors into consideration, although we also confirmed that race and sex could be associated with

differences in the disposition of olanzapine using a meta-analysis (Supplementary figures 3, 4). However, there was insufficient data available to assess the effects of these factors on the clozapine disposition.

Strengths and limitations of the study

The major strengths of this study are that it synthesized the previous studies with standardization of the PK parameters to the C/D ratios, that it clarified the degree of the effect of smoking on the C/D ratios and that it provided standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

On the other hand, there are several limitations to this meta-analysis. The major limitation of this study is the paucity of studies included. This meta-analysis standardized the PK parameters to C/D ratios (ng/mL)/(mg/day), and therefore, only seven studies for olanzapine and four studies for clozapine could be included. We tried to gather information by requesting it from the authors, but 11 studies of olanzapine and 18 studies of clozapine could not be included. This may have led to a selection bias. The second limitation is that this meta-analysis simply divided subjects into smokers and non-smokers, so the amount of smoking was not able to be taken into consideration. It

has been suggested that the smoking-induced changes in hepatic CYP1A2 abundance are dependent on the daily cigarette consumption [36]. Therefore, the differences in the amounts of smoking might have contributed to the variations in the influence of cigarette smoking on the disposition of olanzapine and clozapine among the studies included. Another limitation is that this meta-analysis could not confirm adherence, because none of the studies clearly described the adherence. It was previously reported that up to 80 % of patients with schizophrenia are at least partially nonadherent[37], and this might have affected the results. Finally, the use of co-medications, which may affect the disposition of olanzapine or clozapine, could not be excluded. Six subjects in the study by Laika *et al*., 2010[17] were taking carbamazepine and 21 subjects in the study by Weide *et al*., 2003 were taking carbamazepine or fluvoxamine. These drugs are known to affect the activity of CYP3A4, which is also involved in the metabolism of olanzapine and clozapine.

CONCLUSION

This meta-analysis synthesized previous studies and represented the effects of smoking on the disposition of olanzapine and clozapine in a way that can be used to change the current clinical practices. These results are useful as standards to regulate the

dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status. However, since this meta-analysis standardized the PK parameters, only a few studies were included. Furthermore, this meta-analysis could not consider the amount of smoking or adherence to olanzapine and clozapine treatment. Therefore, additional research is required to establish an administration plan based on the smoking status of patients.

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Contributors

YT reviewed all the abstracts, reviewed all the full papers, performed the statistical analysis and wrote the paper. JS and NY-F reviewed all the abstract titles for relevance, and wrote and reviewed the submitted article.

Competing interests

We declare no competing interests.

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Data sharing statement

There are no additional data available.

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Figure Legends

Figure 1. A flow chart of the study selection process

Abbreviations: C/D, concentration to dose; SD, standard deviation

Figure 2. A forest plot of the primary analyses of olanzapine

Figure 3. A forest plot of the prospective studies of olanzapine

Figure 4. A forest plot of the retrospective studies of olanzapine

Figure 5. A forest plot of the primary analyses of clozapine

Figure 1

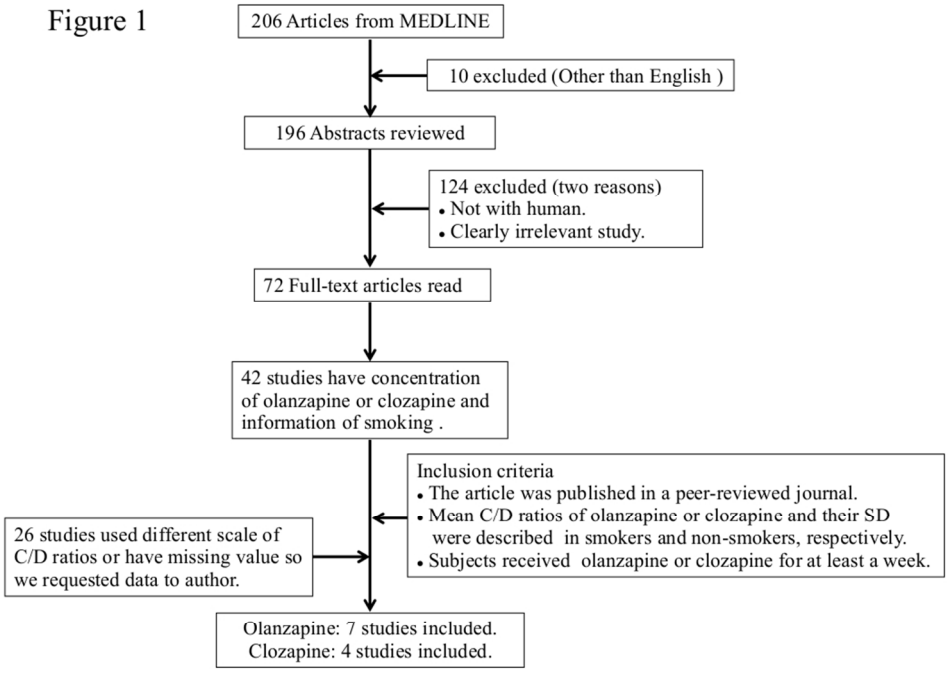

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Figure 2

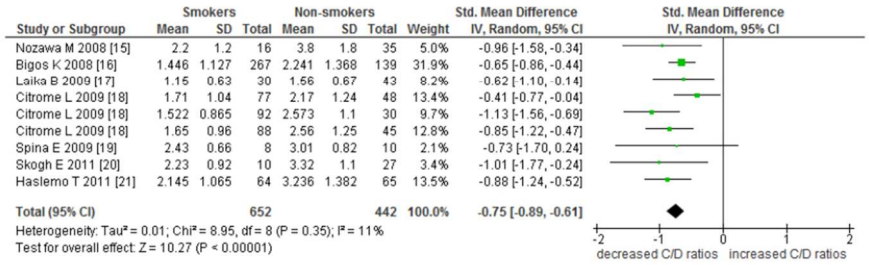

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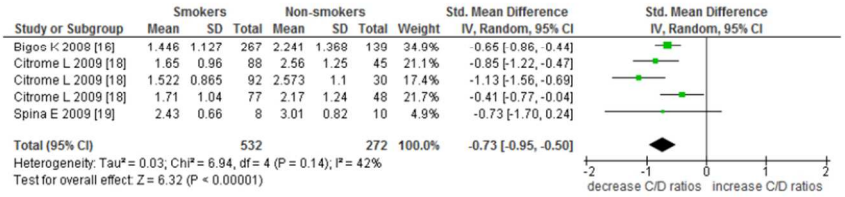
Figure 3



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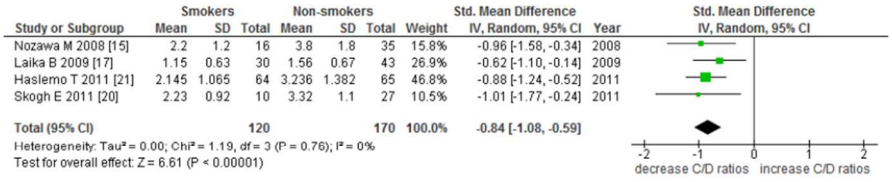

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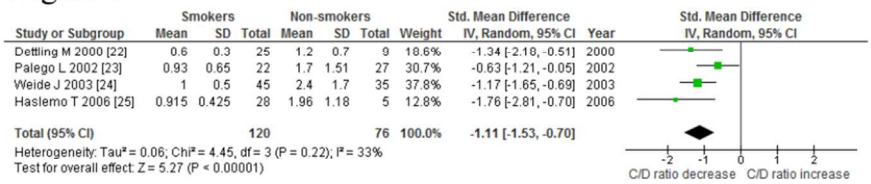
Figure 5



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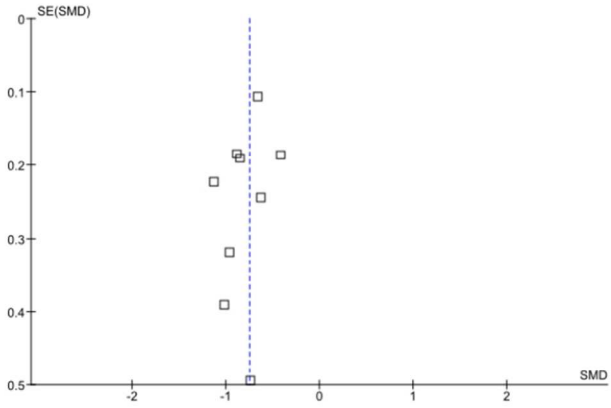
Supplementary Figure 1



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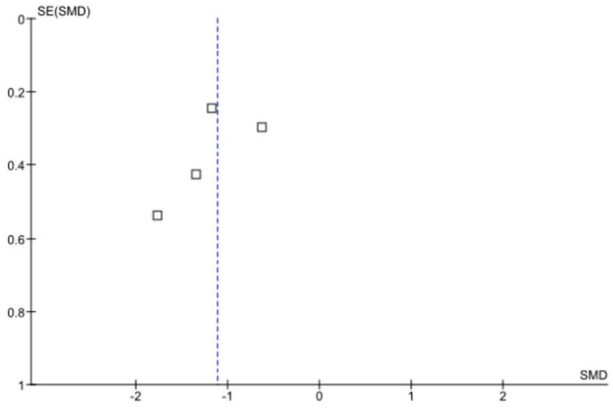
Supplementary Figure 2



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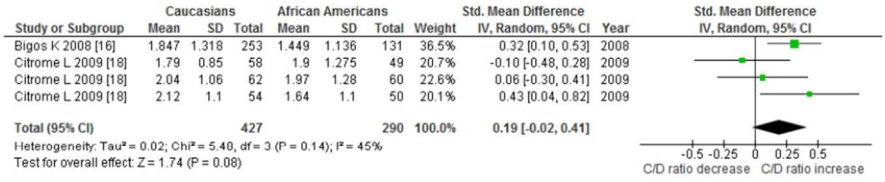
Supplementary Figure 3



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Supplementary Figure 4

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Supplementary Figure legends

Supplementary Figure 1. A funnel plot of the meta-analysis of olanzapine

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 2. A funnel plot of the meta-analysis of clozapine

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 3. A forest plot of the effects of sex on the disposition of olanzapine

Supplementary Figure 4. A forest plot of the effects of race on the disposition of olanzapine

MOOSE Checklist

Article details:

Title: Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

Authors: Yoshiyuki Tsuda, Junji Saruwatari, Norio Yasui-Furukori

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of two commonly used antipsychotics, olanzapine and clozapine. However, no definitive agreement regarding the dose adjustment in clinical practice based on the patient's smoking status has been reached.
√	Hypothesis statement	It may be able to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on the smoking status of the patient by conducting a meta-analysis.
√	Description of study outcomes	The mean concentration to dose (C/D) ratio (ng/ml)/(mg/day) of olanzapine and clozapine
√	Type of exposure or intervention used	Olanzapine or clozapine treatment
√	Type of study designs used	We included both prospective and retrospective studies.
√	Study population	The patients with schizophrenia or other psychiatric diseases who were treated with olanzapine or clozapine
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the investigators, Junji Saruwatari and Norio Yasui-Furukori are included in the author list.
√	Search strategy, including time period included in the synthesis and keywords	MEDLINE from 1946 – August 2012 Six terms in which either 'olanzapine' or 'clozapine' was paired with 'smoking' or 'cigarette' or 'tobacco' or 'smoke'.
√	Databases and registries searched	MEDLINE
√	Search software used, name and version, including special features	We did not employ any search software.
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for additional references.

√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in Figure 1. The citation list is available upon request.
√	Method of addressing articles published in languages other than English	This meta-analysis excluded the article published in languages other than English.
√	Method of handling abstracts and unpublished studies	We did not search unpublished study.
√	Description of any contact with authors	We requested data from the authors if either the C/D ratio of olanzapine or clozapine or the standard deviation (SD) was not described.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies provided mean C/D ratio and the SD values in smokers and non-smokers, respectively.
√	Assessment of confounding	We confirmed that race and sex could be associated with differences in the disposition of olanzapine using a meta-analysis. However, there was insufficient data available to assess the effects of these factors on the clozapine disposition.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Only 7 studies for olanzapine and 4 studies for clozapine could be included. Most studies are low quality, retrospective studies. Only three studies, Bigos KL *et al*.,2008, Citrome L *et al*.,2009, and Spina *et al*.,2009 used the prospective study design.
√	Assessment of heterogeneity	Heterogeneity of the studies was explored with I^2 statistics that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	The weighted mean difference of C/D ratios of olanzapine and clozapine between smokers and non-smokers was calculated by DerSimonian-Laird random effects models.
√	Provision of appropriate tables and graphics	Tabless 1 and2, Figures 1-5, and Supplementary figures 1-4
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figures 2-5
√	Table giving descriptive information for each study included	Tables 1 and 2
√	Results of sensitivity testing	We conducted subgroup analyses of olanzapine. The subgroup analyses (prospective/retrospective studies) also showed results similar to primary

		analyses of olanzapine. In the meta-analyses of clozapine, no subgroup analyses could be conducted because of the small number of patients included in the study.
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates.
Reporting of discussion should include		
√	Quantitative assessment of bias	Publication bias was not shown in both of analyses of olanzapine and clozapine using Egger test and funnel plot. On the other hand, this meta-analysis standardized the pharmacokinetic parameters to C/D ratios (ng/ml)/(mg/day). We tried to gathered information by requesting author, but 11 studies for olanzapine and 18 studies for clozapine could not be included. It may connect to selection bias.
√	Justification for exclusion	We excluded the studies from subjects who have not received olanzapine or clozapine for at least a week.
√	Assessment of quality of included studies	We discussed quality of included studies in discussion section.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Based on the findings of the present study, it was estimated that when 10 mg/day of olanzapine (the usual dose in Japan) was administered to non-smokers, about 13 mg/day should be administered to smokers in order to obtain the equivalent olanzapine concentration. Based on the findings of the present study, it was estimated that when 200 mg/day of clozapine (the usual dose in Japan) was administered to non-smokers, about 360 mg/day should be administered to smokers in order to obtain equivalent clozapine concentration.
√	Generalization of the conclusions	The results of this meta-analysis are useful as standards to regulate dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status. However, this meta-analysis could not take the amount of smoking and adherence into consideration so additional research is required to establish administration plan based on smoking status.
√	Guidelines for future research	Future studies are required to investigate the effect of smoking on olanzapine and clozapine dispositions, while also taking the amount of smoking, adherence, and the other patient's characteristics (e.g., sex, race, genetic polymorphisms) into consideration.
√	Disclosure of funding source	This work was supported by grants from the Japan Research Foundation for Clinical Pharmacology and the Research Group for SCHIZOPHRENIA, and by KAKENHI (Nos. 23510348, 24590652 and 25860117), and in part by a grant from the Smoking Research Foundation..

MOOSE flow chart: Figure 1

BMJ open

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

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Manuscript ID:	bmjopen-2013-004216.R1
Article Type:	Research
Date Submitted by the Author:	24-Dec-2013
Complete List of Authors:	Tsuda, Yoshiyuki; Graduate School of Pharmaceutical Sciences, Kumamoto University, Division of Pharmacology and Therapeutics Saruwatari, Junji; Graduate School of Pharmaceutical Sciences, Kumamoto University, Division of Pharmacology and Therapeutics Yasui-Furukori, Norio; Hirosaki University School of Medicine, Department of Neuropsychiatry
Primary Subject Heading:	Pharmacology and therapeutics
Secondary Subject Heading:	Smoking and tobacco, Mental health, Evidence based practice
Keywords:	Schizophrenia & psychotic disorders < PSYCHIATRY, Adverse events < THERAPEUTICS, MENTAL HEALTH, Toxicity < THERAPEUTICS

TITLE

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotic agents, olanzapine and clozapine

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KEY WORDS

olanzapine, clozapine, smoking, meta-analysis, schizophrenia, disposition

WORD COUNT

3783 words

ABSTRACT

Objective: To clarity the effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine, and to create standards to adjust the doses of these drugs in clinical practice based on the smoking status.

Design: A meta-analysis was conducted by searching MEDLINE, Scopus and the Cochrane Library for relevant prospective and retrospective studies.

Included Studies: We included the studies that investigated the effects of smoking on the concentration to dose (C/D) ratio of olanzapine or clozapine.

Primary outcome measure: The weighted mean difference was calculated using a DerSimonian-Laird random effects model, along with 95% confidence intervals (CI).

Results: Seven association studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders, were included in the meta-analysis of olanzapine. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Four association studies of clozapine were included in the meta-analysis of clozapine,

comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers.

Conclusions: We suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

299 words

ARTICLE SUMMARY

Article focus

- Many studies related to the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but there has been no definitive agreement regarding the dose adjustment needed in clinical practice based on smoking status.
- The meta-analyses of prospective and retrospective studies were conducted to clarify the effects of smoking on the disposition of olanzapine and clozapine and to create standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

Key messages

- The mean difference in the concentration to dose (C/D) ratios of olanzapine between smokers and non-smokers was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration.
- The mean difference in the C/D ratios of clozapine between smokers and

non-smokers was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.

- The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

Strengths and limitations of this study

- The major strength of this study is that it clarifies the effects of smoking on the olanzapine and clozapine concentrations in a large population and provides standards that can be used to regulate the dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status.
- The major limitations of the present study are that we could not use another search engine, e.g., Embase and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked.

Additionally, this meta-analysis standardized pharmacokinetic parameters to C/D ratios, and therefore, only seven studies for olanzapine and four studies for clozapine could be included.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug approved for the treatment of schizophrenia, mania and for preventing the recurrence of bipolar disorders[1]. Olanzapine is a thienobenzodiazepine derivate, which shows potent antagonism at $D_{1\text{-}4}$ dopaminergic receptors, as well as 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[2]. Olanzapine is extensively metabolized in the liver, mainly via cytochrome P450 (CYP) 1A2, but also via CYP2D6, CYP3A4, flavin-containing monooxygenase (FMO) and via glucuronidation[2]. Among these enzymes, CYP1A2 accounts for approximately 50% to 60% of olanzapine metabolism[2].

Clozapine is the prototype atypical antipsychotic, and it belongs to the chemical class of the dibenzodiazepines[1]. Clozapine has much greater antagonistic activity on D_4 than D_2 dopaminergic receptors. It also shows a potent antagonism of 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[1]. Clozapine has been widely used following its introduction, because it induces relatively few extrapyramidal effects, and it shows therapeutic benefits for patients who have failed to respond to other agents[3]. Clozapine is rapidly absorbed, and undergoes extensive hepatic metabolism[4]. Various lines of evidence indicate that CYP1A2 and CYP3A4 play a significant role in both *N*-oxidation and *N*-demethylation of the

compound, whereas CYP2D6 plays a minor role in *N*-demethylation[1 4].

The prevalence of smoking is two- to three-fold higher in patients with schizophrenia than that in the general population, and about 58-88% of patients with schizophrenia are current smokers[5]. Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of many drugs, including olanzapine and clozapine[6].

Citrome *et al*., 2009[7] (n=380) reported that the olanzapine concentrations were significantly lower in smokers with schizophrenia than in non-smokers. Previous clinical studies with small numbers of patients with schizophrenia reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total score than non-smokers[8 9]. Meanwhile, although the relationship between the clozapine concentration and clinical outcome is controversial[10-12], it was also reported that smokers who were treated with clozapine suffered side effects (i.e. auditory hallucinations, hallucinations, hypersalivation, drowsiness, clonic seizures, convulsions and unconsciousness) after smoking cessation[4 13-16].

Many studies about the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but no definitive agreement regarding the dose

adjustment in clinical practice based on the patient's smoking status has been reached. There are several reasons for the slow progress in making the smoking-associated dosage selection; (i) the sample sizes of the previous studies were small; (ii) each study used different pharmacokinetic (PK) parameters [e.g., plasma concentration, plasma concentration to dose (C/D) ratio, clearance (CL)] and the degree of the effect of smoking on the dispositions of olanzapine or clozapine was different between studies. Therefore, a meta-analysis has been needed to overcome the limitations of the previous studies and to determine the degree of the effects of smoking on the disposition of olanzapine and clozapine, in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on smoking status of the patient.

In this study, we performed a meta-analysis of the effects of smoking on the disposition of olanzapine and clozapine.

METHODS

Study selection

A preliminary search of the literature covering the period from 1946 to August 2012 was undertaken to identify publications related to the effects of smoking on the

disposition of olanzapine and clozapine. Electronic databases, including MEDLINE, Scopus and the Cochrane Library, were initially searched using six terms, in which either ‘olanzapine’ or ‘clozapine’ was paired with ‘smoking’ or ‘cigarette’ or ‘tobacco’ or ‘smoke’. We excluded other than English publications, and studies not performed on human participants, after the search. The inclusion criteria were as follows: (i) published in a peer-reviewed journal; (ii) contained the mean C/D ratios (ng/mL)/(mg/day) of olanzapine or clozapine, and their standard deviation (SD) in smokers and non-smokers, respectively, and we requested data from the author(s) if the either the mean C/D ratios or the SD was not described; and (iii) the data were from subjects who had received olanzapine or clozapine for at least a week. In this study, the smokers were defined as the subjects who were currently smoking. Additionally, we divided the selected publications into two groups, i.e. olanzapine and clozapine study groups (Figure 1).

The review and analysis were conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement as a guide[17]. Two researchers (YT and JS) independently searched the literature. Once all the papers had been assessed, any discrepancies in the answers were identified and discussed between the scorers to reach a consensus on the single best option. Any points of assessment on which the scorers could not reach an agreement were resolved by a third scorer (Y-FN).

The data were extracted from each article using a standardized form including the first author, publication year and other information, as described in the following section.

Data extraction

The number of patients, the mean values of the C/D ratios and the SD values of these ratios were extracted for smokers and non-smokers, respectively, from the selected publications. The C/D ratios were standardised to be in the same units, i.e. (ng/mL)/(mg/day). When the values were not described or they were drawn on other scale [e.g., (ng/mL)/(mg/kg)], we asked the author(s) to send us their data in the desired units. We tried to gather information by requesting it from 26 authors. Although five authors responded to our requests, the other 21 studies of olanzapine or clozapine could not be included due to a lack of information (the mean C/D ratios and SD were not available for smokers and non-smokers, respectively, from 14 studies, the SD was not given in four studies, and the mean C/D ratios was described on other scale, i.e. (ng/ml)(mg/kg), in three studies) (Figure 1).

The characteristics of the studies included in this meta-analysis of the effects of smoking on the disposition of olanzapine or clozapine are shown in Tables 1 and 2. We systematically assessed several key points of study quality proposed by the MOOSE

Collaboration[18]. The quality of the included studies is shown in Table 3.

Table 1. The characteristics of the included olanzapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Haslemo T *et al*., 2006	Norway	Retrospective study	51 (16)	34/17	Schizophrenia	Unknown	32.6 ± 9.6
Nozawa M *et al*., 2008	Japan	Retrospective study	51 (16)	34/17	Schizophrenia	DSM-IV	32.6 ± 9.6
Bigos KL *et al*., 2008	USA	Prospective study	406 (267)	289/117	Schizophrenia	DSM-IV	42 ± 7.9
Laika B *et al*., 2009	Germany	Retrospective study	73 (30)	36/37	Schizophrenia, Mood disorder	ICD-10	41.7 ± 14.7
Citrome L *et al*., 2009	USA	Prospective study	380 (257)	265/115	Schizophrenia, Schizoaffective	DSM-IV	18 - 60

					disorder		
					Bipolar disorder,		
Spina E *et al*., 2009	Italy	Prospective study	18 (8)	10/8	Schizoaffective	DSM-IV	39.3 ± 8.6
					disorder		
					Schizophrenia,		
Skogh E *et al*., 2011	Sweden	Retrospective study	37 (10)	25/12	Schizoaffective	DSM-IV	23 – 50
					disorder		
Haslemo T *et al*., 2011	Norway	Retrospective study	129 (64)	0/129	Unknown	Unknown	18 – 40

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.

Table 2. The characteristics of the included clozapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Dettling M *et al*., 2000	Germany	Retrospective study	34 (25)	18/16	Schizophrenia, Bipolar disorder	DSM-III-R	33.7 ± 10.6
Palego L *et al*., 2002	USA	Retrospective study	49 (22)	25/24	Schizophrenia, Schizoaffective disorder	DSM-IV	36.84 ± 1.96 (SE)
Weide J *et al*., 2003	Netherlands	Retrospective study	80 (45)	51/29	Schizophrenia	Unknown	18 - 86
Haslemo T *et al*., 2006	Norway	Retrospective study	33 (28)	21/12	Schizophrenia	Unknown	52 ± 9.0

DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Third Edition-Revised; DSM-IV, Diagnostic and Statistical Manual

of Mental Disorders Fourth Edition.

Table 3. The quality of the included studies

First author	Publication year	Drug treatment	Number of smokers	Diagnostic criteria	Treatment duration	Measurement of blood drug concentration	Sampling scheme	Total score
Haslemo T	2006	Olanzapine	Yes	NA	Yes	Yes	Yes	4
Nozawa M	2008	Olanzapine	Yes	Yes	Yes	Yes	NA	4
Bigos KL	2008	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Laika B	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Citrome L	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Spina E	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Skogh E	2011	Olanzapine	Yes	Yes	Yes	Yes	Yes	5

Haslemo T	2011	Olanzapine	Yes	NA	NA	Yes	Yes	3
Dettling M	2000	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Palego L	2002	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Weide J	2003	Clozapine	Yes	NA	Yes	Yes	Yes	4
Haslemo T	2006	Clozapine	Yes	NA	Yes	Yes	Yes	4

NA, not available.

Statistical analysis

A meta-analysis using the weighted mean difference in the C/D ratios of olanzapine or clozapine between smokers and non-smokers was performed using the Review Manager (RevMan) Version 5.1 for Windows software program (Cochrane Collaboration, http://www.cc-ims.net/RevMan). Cochran's chi-square-based Q-statistic test was applied to assess the between-study heterogeneity. The weighted mean difference was calculated using DerSimonian-Laird random effects models[19], along with 95% confidence intervals (CI), to measure the strength of the association. In this study, we applied the random effects model for the comparisons, which is more conservative because of the possibility that the underlying effect differed across studies and populations. The weighted mean difference was also calculated when the studies were stratified according to the study design, i.e. prospective or retrospective study. We used the I^2 statistic to assess the heterogeneity of the results. Publication bias was assessed by visually examining a funnel plot with asymmetry and formally assessing publication bias with the Egger test[20]. The statistical significance level for all analyses was set at a two-sided value of $p<0.05$.

RESULTS

Olanzapine: Search results and study characteristics

Eight studies of olanzapine[7 21-27] met our criteria (Figure 1). The studies included in this analysis for olanzapine are listed in Table 1. Since the study by Citrome *et al*., 2009[7] was derived from a randomized clinical trial of 10, 20, and 40 mg as the daily olanzapine dose in patients with schizophrenia or schizoaffective disorder, we divided its populations into three groups according to the respective olanzapine doses. Since the study by Spina *et al*., 2009[25] focused on the effects of valproate on the olanzapine plasma concentrations, so we extracted the C/D ratios of olanzapine at baseline (before taking valproate). The study by Haslemo *et al*., 2011[27] focused on the effects of contraceptives on the serum concentration of olanzapine among female patients who were treated either with olanzapine alone or the combination of estradiol-containing contraceptives, so we requested the C/D ratios in subjects not using any contraceptives that can affect the CYP1A2 activity.

Primary analyses of olanzapine

The weighted mean difference was derived from all studies, comprising a total of 1134 patients (683 smokers and 451 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in

non-smokers (p<0.00001) (Figure 2), and the mean difference was -0.83 (ng/mL)/(mg/day) (95% CI: -1.04 to -0.63). Although there was no significant publication bias (p=0.26), significant heterogeneity was observed (I^2=50, p=0.04). Since we included two studies by the same authors, we excluded the older study (Haslemo *et al*., 2006[21]) in the subsequent analyses to reduce the heterogeneity.

The analysis from the seven studies showed that there was no significant heterogeneity among the mean differences (I^2=11%, p= 0.35) (Figure 3a). The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 3a), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI: -0.89 to -0.61). No significant publication bias was shown using the Egger test in the studies of olanzapine (p=0.282). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 1).

Subgroup analyses of olanzapine

Prospective studies

We conducted subgroup analyses to confirm the precision of the primary

analyses. Of the seven included studies of olanzapine, three were prospective studies, while four were retrospective studies. In the prospective studies (532 smokers and 272 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3b), and the mean difference was -0.73 (ng/mL)/(mg/day) (95% CI: -0.95 to -0.50).

Retrospective studies

In the retrospective studies (120 smokers and 170 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3c), and the mean difference was -0.84 (ng/mL)/(mg/day) (95% CI: -1.08 to -0.59).

Clozapine: Search results and study characteristics

Four studies regarding the clozapine disposition[21 28-30] met our criteria, all of which were retrospective studies (Figure 1). The clozapine studies included in this analysis are listed in Table 2.

Analyses of clozapine

There was no significant heterogeneity among the mean differences (I^2=33%,

p=0.22) (Figure 4). The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 4), and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). No significant bias was shown using the Egger test for the clozapine studies (p=0.436). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 2).

DISCUSSION

Smoking is a well-known cause of significant drug interactions in humans[31-33]. The polyaromatic hydrocarbons in cigarette smoke are known to induce CYP1A2[34], and therefore, cigarette smoking can affect the disposition of drugs that are metabolized by CYP1A2, such as olanzapine and clozapine. The prevalence of current smokers is higher in patients with schizophrenia than that in the general population[5]. However, at present, there is no definitive data regarding the dose adjustments of olanzapine and clozapine in clinical practice based on the patient's smoking status. This is the first meta-analysis to clarify the effects of smoking on the disposition of these drugs.

Olanzapine

In the meta-analysis of olanzapine, 1094 patients (652 smokers and 442 non-smokers) from seven clinical studies of olanzapine were evaluated. The results showed that the C/D ratio of olanzapine was 0.75 (ng/mL)/(mg/day) lower in smokers than in non-smokers. The subgroup analyses (prospective/retrospective studies) also showed similar results. Approximately 85% of the oral olanzapine dose is absorbed, but as about 40% is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60%[1]. The mean half-life, mean apparent drug plasma CL and mean apparent volume of distribution of olanzapine were 33 hours, 26 L/h and 1150 L in healthy individuals[35]. Previous clinical studies demonstrated that the C/D ratio of olanzapine significantly correlated with a decrease in the Brief Psychiatric Rating Scale[8 9]. The association between the clinical outcome and the plasma olanzapine concentration is clearly curvilinear, with clinical efficacy being approximately associated with a plasma olanzapine concentration range of 20-50 ng/mL[1]. Bigos *et al*., 2008[23] (n=523) analyzed the population pharmacokinetics of olanzapine, and they determined that sex, smoking and race contribute to the variability in olanzapine clearance. The study also demonstrated that smoking increased the olanzapine clearance by 55%, while also incorporating other confounding factors. Based on the findings of the present study, it

was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 7/10 in non-smokers compared with smokers.

Clozapine

In the meta-analysis of clozapine, 196 patients (smokers: 120, non-smokers: 76) from four clinical studies were evaluated. The results showed that the C/D ratio of clozapine was 1.11 (ng/mL)/(mg/day) lower in smokers than in non-smokers. After oral administration of clozapine, the drug is rapidly absorbed. Only 27-50% of the dose reaches the systemic circulation unchanged, because of extensive first-pass metabolism[1]. There is a wide inter-patitent variability in PK parameters of clozapine[1]. The mean half-life of clozapine ranges from 9 to 17 hours[1]. The plasma CL of clozapine was reported to be between 9 and 53 L/hour, and the volume of distribution of clozapine was between 2 and 7 L/kg[1]. The steady-state plasma concentrations of clozapine are reached after 7-10 days of dosing[1]. The relationship between the clozapine concentration and clinical outcome is controversial. According to the study by Spina *et al.*, 2000[11], a

receiver operating characteristics analysis showed that a clozapine concentration cut-off value of 350 ng/mL distinguished responders and non-responders with a sensitivity of 72% and a specificity of 70%. On the other hand, it has been suggested that the clozapine concentration does not correlate with the decrease in the Brief Psychiatric Rating Scale[10 12].

A recent review summarized the previous studies regarding the relationships between the clozapine concentrations and clinical response, and suggested that clozapine levels above 250-400 ng/mL are associated with an increased chance of a clinical response [36]. Moreover, clozapine doses exceeding 500-600 mg/day of clozapine could carry an increased risk of seizures[36]. Because the smokers who were treated with clozapine were reported to suffer serious central nervous side effects after smoking cessation[4 13-16], it is necessary to regulate the clozapine dosage carefully when smokers stop smoking or decrease the amount of smoking. Li *et al*., 2012[36] applied nonlinear mixed-effect modelling to characterize the pharmacokinetics of clozapine in Chinese patients. In the final model, sex and the smoking status were identified as significant covariates for the clearance of clozapine and norclozapine[36], and smokers had a 1.45-fold higher clearance of clozapine than non-smokers[36]. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual

doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of clozapine should be reduced by 1/2 in non-smokers compared with smokers.

Other factors affecting the disposition of olanzapine and clozapine

Many previous clinical studies reported that sex, race, age, co-medication and the genotype could affect the disposition of olanzapine and clozapine[23 37-47]. Since estrogen is known to inhibit the activity of CYP1A2[23], it is not surprising that the clearance of olanzapine and clozapine was reported to be lower in females than in males[23]. Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as ethynilestradiol, fluozetine, fluvoxamine, fluoxetine, fluxoxamine, paroxetine, sertraline, valproate and venlafaxine, were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4[27 41 43 45 48]. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4[41 45 48 49]. Race is known to be associated with variability in the

CYP1A2 activity. Bigos *et al*., 2008[23] reported that African Americans cleared olanzapine faster than did other races (i.e., Caucasians, Asians and Native Americans). Moreover, many genetic polymorphisms were reported to affect to the disposition of olanzapine and clozapine. A recent review suggested that *UGT1A4*3*, *CYP1A2* rs2472297, *FMO3* K158-G308, *FMO1*6*, *FMO1* rs7877 and *CYP3A43* rs472660 polymorphisms all influence the olanzapine metabolism[50]. Regarding clozapine, Lee *et al*., 2012[44] showed that *CYP1A2* rs2069521 and rs2069522 polymorphisms were significantly associated with the C/D ratio of clozapine, and *CYP2D6* rs1135840 was associated with the ratio of norclozapine and clozapine. Nevertheless, in the present study, there was insufficient data available to assess the effects of these factors on the disposition of olanzapine or clozapine. Moreover, the influence of smoking on the disposition of olanzapine and clozapine might be different among different patient populations (e.g., the elderly, females, different diagnostic groups), but we could not conduct a meta-analysis for these populations.

Strengths and limitations of the study

The major strengths of this study are that it synthesized the previous studies with standardization of the PK parameters to the C/D ratios, that it clarified the degree

of the effect of smoking on the C/D ratios and that it provided standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

On the other hand, there are several limitations to this meta-analysis. The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked. This meta-analysis standardized the PK parameters to C/D ratios (ng/mL)/(mg/day), and therefore, only seven studies for olanzapine and four studies for clozapine could be included. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies.

Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo *et al*., 2006[21]) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias. Furthermore, we included the three results from Citrome *et a*l., 2009[7] independently, and therefore, should verify the correlation of these results using a random intercept in the mixed effects meta-analysis. When the three results were separately included in the meta-analysis, the weighted differences were not significantly different among the analyses (Supplementary figure 3). However, we could not apply the random intercept in the mixed effects meta-analysis, because we used the Review Manager (RevMan) software program, which lacks this function for the analysis. In previous studies, the sum concentrations of clozapine and its metabolite, norclozapine, and the norclozapine to clozapine ratio, were also used as a clinical outcome and an index of metabolic activity, respectively[1]. However, we could not use these parameters for the present meta-analysis, because we used only the clozapine concentration to dose ratio in order to be able to include as many studies as possible and to develop simple standards that can be used in clinical practice.

The other limitation is that this meta-analysis simply divided subjects into smokers and non-smokers, so the amount of smoking was not able to be taken into consideration. It has been suggested that the smoking-induced changes in hepatic CYP1A2 abundance are dependent on the daily cigarette consumption[51]. Therefore, the differences in the amounts of smoking might have contributed to the variations in the influence of cigarette smoking on the disposition of olanzapine and clozapine among the studies included. Additionally, this meta-analysis could not confirm patient adherence. It was previously reported that up to 80 % of patients with schizophrenia are at least partially nonadherent[52], and this might have affected the results. Although we included the studies that described that the subjects had taken the drug for at least a week, we could not obtain any information regarding the adherence, because none of the studies clearly described this information. Finally, the use of co-medications, which may affect the disposition of olanzapine or clozapine, could not be excluded. Six subjects in the study by Laika *et al*., 2010[24] were taking carbamazepine and 21 subjects in the study by Weide *et al*., 2003 were taking carbamazepine or fluvoxamine. These drugs are known to affect the activity of CYP1A2 and/or CYP3A4, which is also involved in the metabolism of olanzapine and clozapine.

CONCLUSION

This meta-analysis synthesized previous studies and represented the effects of smoking on the disposition of olanzapine and clozapine in a way that can be used to change the current clinical practices. Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2 in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

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Contributors

YT reviewed all the abstracts, reviewed all the full papers, performed the statistical analysis and wrote the paper. JS and NY-F reviewed all of the abstracts and full papers for relevance, and wrote and reviewed the submitted article.

Competing interests

We declare no competing interests.

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Data sharing statement

There are no additional data available.

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Figure Legends

Figure 1. A flow chart of the study selection process

Abbreviations: C/D, concentration to dose; SD, standard deviation

Figure 2. Forest plot olanzapine (n=8)

Figure 3. Forest plot (a) olanzapine study (n=7) (b) prospective olanzapine study (n=3)

(c) retrospective olanzapine study (n=4)

Figure 4. Forest plot clozapine (n=4)

Supplementary Figure legends

Supplementary Figure 1. The funnel plot of olanzapine (n=7) (the study by Citrome *et al*., 2009 is represented by three data points in this figure)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 2. The funnel plot of clozapine (n=4)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 3. The forest plot of olanzapine (n=5) (a) including only the data for 10 mg olanzapine reported by Citrome *et al*., 2009 (b) including only the data for 20 mg olanzapine reported by Citrome *et al*., 2009 and (c) including only the data for 40 mg olanzapine reported by Citrome *et al*., 2009

TITLE

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotic agents, olanzapine and clozapine

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ABSTRACT

Objective: To clarity the effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine, and to create standards to adjust the doses of these drugs in clinical practice based on the smoking status.

Design: A meta-analysis was conducted by searching MEDLINE, Scopus and the Cochrane Library for relevant prospective and retrospective studies.

Included Studies: We included the studies that investigated the effects of smoking on the concentration to dose (C/D) ratio of olanzapine or clozapine.

Primary outcome measure: The weighted mean difference was calculated using a DerSimonian-Laird random effects model, along with 95% confidence intervals (CI).

Results: Seven association studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders, were included in the meta-analysis of olanzapine. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Four association studies of clozapine were included in the meta-analysis of clozapine,

comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers.

Conclusions: We suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

299 words

ARTICLE SUMMARY

Article focus

- Many studies related to the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but there has been no definitive agreement regarding the dose adjustment needed in clinical practice based on smoking status.
- The meta-analyses of prospective and retrospective studies were conducted to clarify the effects of smoking on the disposition of olanzapine and clozapine and to create standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

Key messages

- The mean difference in the concentration to dose (C/D) ratios of olanzapine between smokers and non-smokers was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration.
- The mean difference in the C/D ratios of clozapine between smokers and

non-smokers was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.

- The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

Strengths and limitations of this study

- The major strength of this study is that it clarifies the effects of smoking on the olanzapine and clozapine concentrations in a large population and provides standards that can be used to regulate the dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status.
- The major limitations of the present study are that we could not use another search engine, e.g., Embase and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked.

Additionally, this meta-analysis standardized pharmacokinetic parameters to C/D ratios, and therefore, only seven studies for olanzapine and four studies for clozapine could be included.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug approved for the treatment of schizophrenia, mania and for preventing the recurrence of bipolar disorders[1]. Olanzapine is a thienobenzodiazepine derivate, which shows potent antagonism at $D_{1\text{-}4}$ dopaminergic receptors, as well as 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[2]. Olanzapine is extensively metabolized in the liver, mainly via cytochrome P450 (CYP) 1A2, but also via CYP2D6, CYP3A4, flavin-containing monooxygenase (FMO) and via glucuronidation[2]. Among these enzymes, CYP1A2 accounts for approximately 50% to 60% of olanzapine metabolism[2].

Clozapine is the prototype atypical antipsychotic, and it belongs to the chemical class of the dibenzodiazepines[1]. Clozapine has much greater antagonistic activity on D_4 than D_2 dopaminergic receptors. It also shows a potent antagonism of 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[1]. Clozapine has been widely used following its introduction, because it induces relatively few extrapyramidal effects, and it shows therapeutic benefits for patients who have failed to respond to other agents[3]. Clozapine is rapidly absorbed, and undergoes extensive hepatic metabolism[4]. Various lines of evidence indicate that CYP1A2 and CYP3A4 play a significant role in both *N*-oxidation and *N*-demethylation of the

compound, whereas CYP2D6 plays a minor role in *N*-demethylation[1 4].

The prevalence of smoking is two- to three-fold higher in patients with schizophrenia than that in the general population, and about 58-88% of patients with schizophrenia are current smokers[5]. Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of many drugs, including olanzapine and clozapine[6].

Citrome *et al*., 2009[7] (n=380) reported that the olanzapine concentrations were significantly lower in smokers with schizophrenia than in non-smokers. Previous clinical studies with small numbers of patients with schizophrenia reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total score than non-smokers[8 9]. Meanwhile, although the relationship between the clozapine concentration and clinical outcome is controversial[10-12], it was also reported that smokers who were treated with clozapine suffered side effects (i.e. auditory hallucinations, hallucinations, hypersalivation, drowsiness, clonic seizures, convulsions and unconsciousness) after smoking cessation[4 13-16].

Many studies about the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but no definitive agreement regarding the dose

adjustment in clinical practice based on the patient's smoking status has been reached. There are several reasons for the slow progress in making the smoking-associated dosage selection; (i) the sample sizes of the previous studies were small; (ii) each study used different pharmacokinetic (PK) parameters [e.g., plasma concentration, plasma concentration to dose (C/D) ratio, clearance (CL)] and the degree of the effect of smoking on the dispositions of olanzapine or clozapine was different between studies. Therefore, a meta-analysis has been needed to overcome the limitations of the previous studies and to determine the degree of the effects of smoking on the disposition of olanzapine and clozapine, in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on smoking status of the patient.

In this study, we performed a meta-analysis of the effects of smoking on the disposition of olanzapine and clozapine.

METHODS

Study selection

A preliminary search of the literature covering the period from 1946 to August 2012 was undertaken to identify publications related to the effects of smoking on the

disposition of olanzapine and clozapine. Electronic databases, including MEDLINE, Scopus and the Cochrane Library, were initially searched using six terms, in which either 'olanzapine' or 'clozapine' was paired with 'smoking' or 'cigarette' or 'tobacco' or 'smoke'. We excluded other than English publications, and studies not performed on human participants, after the search. The inclusion criteria were as follows: (i) published in a peer-reviewed journal; (ii) contained the mean C/D ratios (ng/mL)/(mg/day) of olanzapine or clozapine, and their standard deviation (SD) in smokers and non-smokers, respectively, and we requested data from the author(s) if the either the mean C/D ratios or the SD was not described; and (iii) the data were from subjects who had received olanzapine or clozapine for at least a week. In this study, the smokers were defined as the subjects who were currently smoking. Additionally, we divided the selected publications into two groups, i.e. olanzapine and clozapine study groups (Figure 1).

The review and analysis were conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement as a guide[17]. Two researchers (YT and JS) independently searched the literature. Once all the papers had been assessed, any discrepancies in the answers were identified and discussed between the scorers to reach a consensus on the single best option. Any points of assessment on which the scorers could not reach an agreement were resolved by a third scorer (Y-FN).

The data were extracted from each article using a standardized form including the first author, publication year and other information, as described in the following section.

Data extraction

The number of patients, the mean values of the C/D ratios and the SD values of these ratios were extracted for smokers and non-smokers, respectively, from the selected publications. The C/D ratios were standardised to be in the same units, i.e. (ng/mL)/(mg/day). When the values were not described or they were drawn on other scale [e.g., (ng/mL)/(mg/kg)], we asked the author(s) to send us their data in the desired units. We tried to gather information by requesting it from 26 authors. Although five authors responded to our requests, the other 21 studies of olanzapine or clozapine could not be included due to a lack of information (the mean C/D ratios and SD were not available for smokers and non-smokers, respectively, from 14 studies, the SD was not given in four studies, and the mean C/D ratios was described on other scale, i.e. (ng/ml)(mg/kg), in three studies) (Figure 1).

The characteristics of the studies included in this meta-analysis of the effects of smoking on the disposition of olanzapine or clozapine are shown in Tables 1 and 2. We systematically assessed several key points of study quality proposed by the MOOSE

Collaboration[18]. The quality of the included studies is shown in Table 3.

Table 1. The characteristics of the included olanzapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Haslemo T *et al*., 2006	Norway	Retrospective study	51 (16)	34/17	Schizophrenia	Unknown	32.6 ± 9.6
Nozawa M *et al*., 2008	Japan	Retrospective study	51 (16)	34/17	Schizophrenia	DSM-IV	32.6 ± 9.6
Bigos KL *et al*., 2008	USA	Prospective study	406 (267)	289/117	Schizophrenia	DSM-IV	42 ± 7.9
Laika B *et al*., 2009	Germany	Retrospective study	73 (30)	36/37	Schizophrenia, Mood disorder	ICD-10	41.7 ± 14.7
Citrome L *et al*., 2009	USA	Prospective study	380 (257)	265/115	Schizophrenia, Schizoaffective	DSM-IV	18 - 60

					disorder		
Spina E *et al*., 2009	Italy	Prospective study	18 (8)	10/8	Bipolar disorder, Schizoaffective disorder	DSM-IV	39.3 ± 8.6
Skogh E *et al*., 2011	Sweden	Retrospective study	37 (10)	25/12	Schizophrenia, Schizoaffective disorder	DSM-IV	23 – 50
Haslemo T *et al*., 2011	Norway	Retrospective study	129 (64)	0/129	Unknown	Unknown	18 – 40

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.

Table 2. The characteristics of the included clozapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Dettling M *et al*., 2000	Germany	Retrospective study	34 (25)	18/16	Schizophrenia, Bipolar disorder	DSM-III-R	33.7 ± 10.6
Palego L *et al*., 2002	USA	Retrospective study	49 (22)	25/24	Schizophrenia, Schizoaffective disorder	DSM-IV	36.84 ± 1.96 (SE)
Weide J *et al*., 2003	Netherlands	Retrospective study	80 (45)	51/29	Schizophrenia	Unknown	18 - 86
Haslemo T *et al*., 2006	Norway	Retrospective study	33 (28)	21/12	Schizophrenia	Unknown	52 ± 9.0

DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Third Edition-Revised; DSM-IV, Diagnostic and Statistical Manual

of Mental Disorders Fourth Edition.

Table 3. The quality of the included studies

First author	Publication year	Drug treatment	Number of smokers	Diagnostic criteria	Treatment duration	Measurement of blood drug concentration	Sampling scheme	Total score
Haslemo T	2006	Olanzapine	Yes	NA	Yes	Yes	Yes	4
Nozawa M	2008	Olanzapine	Yes	Yes	Yes	Yes	NA	4
Bigos KL	2008	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Laika B	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Citrome L	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Spina E	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Skogh E	2011	Olanzapine	Yes	Yes	Yes	Yes	Yes	5

Haslemo T	2011	Olanzapine	Yes	NA	NA	Yes	Yes	3
Dettling M	2000	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Palego L	2002	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Weide J	2003	Clozapine	Yes	NA	Yes	Yes	Yes	4
Haslemo T	2006	Clozapine	Yes	NA	Yes	Yes	Yes	4

NA, not available.

Statistical analysis

A meta-analysis using the weighted mean difference in the C/D ratios of olanzapine or clozapine between smokers and non-smokers was performed using the Review Manager (RevMan) Version 5.1 for Windows software program (Cochrane Collaboration, http://www.cc-ims.net/RevMan). Cochran's chi-square-based Q-statistic test was applied to assess the between-study heterogeneity. The weighted mean difference was calculated using DerSimonian-Laird random effects models[19], along with 95% confidence intervals (CI), to measure the strength of the association. In this study, we applied the random effects model for the comparisons, which is more conservative because of the possibility that the underlying effect differed across studies and populations. The weighted mean difference was also calculated when the studies were stratified according to the study design, i.e. prospective or retrospective study. We used the I^2 statistic to assess the heterogeneity of the results. Publication bias was assessed by visually examining a funnel plot with asymmetry and formally assessing publication bias with the Egger test[20]. The statistical significance level for all analyses was set at a two-sided value of $p<0.05$.

RESULTS

Olanzapine: Search results and study characteristics

Eight studies of olanzapine[7 21-27] met our criteria (Figure 1). The studies included in this analysis for olanzapine are listed in Table 1. Since the study by Citrome *et al*., 2009[7] was derived from a randomized clinical trial of 10, 20, and 40 mg as the daily olanzapine dose in patients with schizophrenia or schizoaffective disorder, we divided its populations into three groups according to the respective olanzapine doses. Since the study by Spina *et al*., 2009[25] focused on the effects of valproate on the olanzapine plasma concentrations, so we extracted the C/D ratios of olanzapine at baseline (before taking valproate). The study by Haslemo *et al*., 2011[27] focused on the effects of contraceptives on the serum concentration of olanzapine among female patients who were treated either with olanzapine alone or the combination of estradiol-containing contraceptives, so we requested the C/D ratios in subjects not using any contraceptives that can affect the CYP1A2 activity.

Primary analyses of olanzapine

The weighted mean difference was derived from all studies, comprising a total of 1134 patients (683 smokers and 451 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in

non-smokers (p<0.00001) (Figure 2), and the mean difference was -0.83 (ng/mL)/(mg/day) (95% CI: -1.04 to -0.63). Although there was no significant publication bias (p=0.26), significant heterogeneity was observed (I^2=50, p=0.04). Since we included two studies by the same authors, we excluded the older study (Haslemo *et al*., 2006[21]) in the subsequent analyses to reduce the heterogeneity.

The analysis from the seven studies showed that there was no significant heterogeneity among the mean differences (I^2=11%, p= 0.35) (Figure 3a). The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 3a), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI: -0.89 to -0.61). No significant publication bias was shown using the Egger test in the studies of olanzapine (p=0.282). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 1).

Subgroup analyses of olanzapine

Prospective studies

We conducted subgroup analyses to confirm the precision of the primary

analyses. Of the seven included studies of olanzapine, three were prospective studies, while four were retrospective studies. In the prospective studies (532 smokers and 272 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3b), and the mean difference was -0.73 (ng/mL)/(mg/day) (95% CI: -0.95 to -0.50).

Retrospective studies

In the retrospective studies (120 smokers and 170 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3c), and the mean difference was -0.84 (ng/mL)/(mg/day) (95% CI: -1.08 to -0.59).

Clozapine: Search results and study characteristics

Four studies regarding the clozapine disposition[21 28-30] met our criteria, all of which were retrospective studies (Figure 1). The clozapine studies included in this analysis are listed in Table 2.

Analyses of clozapine

There was no significant heterogeneity among the mean differences (I^2=33%,

p=0.22) (Figure 4). The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 4), and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). No significant bias was shown using the Egger test for the clozapine studies (p=0.436). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 2).

DISCUSSION

Smoking is a well-known cause of significant drug interactions in humans[31-33]. The polyaromatic hydrocarbons in cigarette smoke are known to induce CYP1A2[34], and therefore, cigarette smoking can affect the disposition of drugs that are metabolized by CYP1A2, such as olanzapine and clozapine. The prevalence of current smokers is higher in patients with schizophrenia than that in the general population[5]. However, at present, there is no definitive data regarding the dose adjustments of olanzapine and clozapine in clinical practice based on the patient's smoking status. This is the first meta-analysis to clarify the effects of smoking on the disposition of these drugs.

Olanzapine

In the meta-analysis of olanzapine, 1094 patients (652 smokers and 442 non-smokers) from seven clinical studies of olanzapine were evaluated. The results showed that the C/D ratio of olanzapine was 0.75 (ng/mL)/(mg/day) lower in smokers than in non-smokers. The subgroup analyses (prospective/retrospective studies) also showed similar results. Approximately 85% of the oral olanzapine dose is absorbed, but as about 40% is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60%[1]. The mean half-life, mean apparent drug plasma CL and mean apparent volume of distribution of olanzapine were 33 hours, 26 L/h and 1150 L in healthy individuals[35]. Previous clinical studies demonstrated that the C/D ratio of olanzapine significantly correlated with a decrease in the Brief Psychiatric Rating Scale[8 9]. The association between the clinical outcome and the plasma olanzapine concentration is clearly curvilinear, with clinical efficacy being approximately associated with a plasma olanzapine concentration range of 20-50 ng/mL[1]. Bigos *et al.*, 2008[23] (n=523) analyzed the population pharmacokinetics of olanzapine, and they determined that sex, smoking and race contribute to the variability in olanzapine clearance. The study also demonstrated that smoking increased the olanzapine clearance by 55%, while also incorporating other confounding factors. Based on the findings of the present study, it

was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 7/10 in non-smokers compared with smokers.

Clozapine

In the meta-analysis of clozapine, 196 patients (smokers: 120, non-smokers: 76) from four clinical studies were evaluated. The results showed that the C/D ratio of clozapine was 1.11 (ng/mL)/(mg/day) lower in smokers than in non-smokers. After oral administration of clozapine, the drug is rapidly absorbed. Only 27-50% of the dose reaches the systemic circulation unchanged, because of extensive first-pass metabolism[1]. There is a wide inter-patitent variability in PK parameters of clozapine[1]. The mean half-life of clozapine ranges from 9 to 17 hours[1]. The plasma CL of clozapine was reported to be between 9 and 53 L/hour, and the volume of distribution of clozapine was between 2 and 7 L/kg[1]. The steady-state plasma concentrations of clozapine are reached after 7-10 days of dosing[1]. The relationship between the clozapine concentration and clinical outcome is controversial. According to the study by Spina *et al.*, 2000[11], a

receiver operating characteristics analysis showed that a clozapine concentration cut-off value of 350 ng/mL distinguished responders and non-responders with a sensitivity of 72% and a specificity of 70%. On the other hand, it has been suggested that the clozapine concentration does not correlate with the decrease in the Brief Psychiatric Rating Scale[10 12].

A recent review summarized the previous studies regarding the relationships between the clozapine concentrations and clinical response, and suggested that clozapine levels above 250-400 ng/mL are associated with an increased chance of a clinical response [36]. Moreover, clozapine doses exceeding 500-600 mg/day of clozapine could carry an increased risk of seizures[36]. Because the smokers who were treated with clozapine were reported to suffer serious central nervous side effects after smoking cessation[4 13-16], it is necessary to regulate the clozapine dosage carefully when smokers stop smoking or decrease the amount of smoking. Li *et al*., 2012[36] applied nonlinear mixed-effect modelling to characterize the pharmacokinetics of clozapine in Chinese patients. In the final model, sex and the smoking status were identified as significant covariates for the clearance of clozapine and norclozapine[36], and smokers had a 1.45-fold higher clearance of clozapine than non-smokers[36]. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual

doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of clozapine should be reduced by 1/2 in non-smokers compared with smokers.

Other factors affecting the disposition of olanzapine and clozapine

Many previous clinical studies reported that sex, race, age, co-medication and the genotype could affect the disposition of olanzapine and clozapine[23 37-47]. Since estrogen is known to inhibit the activity of CYP1A2[23], it is not surprising that the clearance of olanzapine and clozapine was reported to be lower in females than in males[23]. Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as ethynilestradiol, fluozetine, fluvoxamine, fluoxetine, fluxoxamine, paroxetine, sertraline, valproate and venlafaxine, were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4[27 41 43 45 48]. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4[41 45 48 49]. Race is known to be associated with variability in the

CYP1A2 activity. Bigos *et al*., 2008[23] reported that African Americans cleared olanzapine faster than did other races (i.e., Caucasians, Asians and Native Americans). Moreover, many genetic polymorphisms were reported to affect to the disposition of olanzapine and clozapine. A recent review suggested that *UGT1A4*3*, *CYP1A2* rs2472297, *FMO3* K158-G308, *FMO1*6*, *FMO1* rs7877 and *CYP3A43* rs472660 polymorphisms all influence the olanzapine metabolism[50]. Regarding clozapine, Lee *et al*., 2012[44] showed that *CYP1A2* rs2069521 and rs2069522 polymorphisms were significantly associated with the C/D ratio of clozapine, and *CYP2D6* rs1135840 was associated with the ratio of norclozapine and clozapine. Nevertheless, in the present study, there was insufficient data available to assess the effects of these factors on the disposition of olanzapine or clozapine. Moreover, the influence of smoking on the disposition of olanzapine and clozapine might be different among different patient populations (e.g., the elderly, females, different diagnostic groups), but we could not conduct a meta-analysis for these populations.

Strengths and limitations of the study

The major strengths of this study are that it synthesized the previous studies with standardization of the PK parameters to the C/D ratios, that it clarified the degree

of the effect of smoking on the C/D ratios and that it provided standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

On the other hand, there are several limitations to this meta-analysis. The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked. This meta-analysis standardized the PK parameters to C/D ratios (ng/mL)/(mg/day), and therefore, only seven studies for olanzapine and four studies for clozapine could be included. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies.

Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo *et al*., 2006[21]) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias. Furthermore, we included the three results from Citrome *et a*l., 2009[7] independently, and therefore, should verify the correlation of these results using a random intercept in the mixed effects meta-analysis. When the three results were separately included in the meta-analysis, the weighted differences were not significantly different among the analyses (Supplementary figure 3). However, we could not apply the random intercept in the mixed effects meta-analysis, because we used the Review Manager (RevMan) software program, which lacks this function for the analysis. In previous studies, the sum concentrations of clozapine and its metabolite, norclozapine, and the norclozapine to clozapine ratio, were also used as a clinical outcome and an index of metabolic activity, respectively[1]. However, we could not use these parameters for the present meta-analysis, because we used only the clozapine concentration to dose ratio in order to be able to include as many studies as possible and to develop simple standards that can be used in clinical practice.

The other limitation is that this meta-analysis simply divided subjects into smokers and non-smokers, so the amount of smoking was not able to be taken into consideration. It has been suggested that the smoking-induced changes in hepatic CYP1A2 abundance are dependent on the daily cigarette consumption[51]. Therefore, the differences in the amounts of smoking might have contributed to the variations in the influence of cigarette smoking on the disposition of olanzapine and clozapine among the studies included. Additionally, this meta-analysis could not confirm patient adherence. It was previously reported that up to 80 % of patients with schizophrenia are at least partially nonadherent[52], and this might have affected the results. Although we included the studies that described that the subjects had taken the drug for at least a week, we could not obtain any information regarding the adherence, because none of the studies clearly described this information. Finally, the use of co-medications, which may affect the disposition of olanzapine or clozapine, could not be excluded. Six subjects in the study by Laika *et al*., 2010[24] were taking carbamazepine and 21 subjects in the study by Weide *et al*., 2003 were taking carbamazepine or fluvoxamine. These drugs are known to affect the activity of CYP1A2 and/or CYP3A4, which is also involved in the metabolism of olanzapine and clozapine.

CONCLUSION

This meta-analysis synthesized previous studies and represented the effects of smoking on the disposition of olanzapine and clozapine in a way that can be used to change the current clinical practices. Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and 1/2 in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

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Contributors

YT reviewed all the abstracts, reviewed all the full papers, performed the statistical analysis and wrote the paper. JS and NY-F reviewed all of the abstracts and full papers for relevance, and wrote and reviewed the submitted article.

Competing interests

We declare no competing interests.

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Data sharing statement

There are no additional data available.

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Figure Legends

Figure 1. A flow chart of the study selection process

Abbreviations: C/D, concentration to dose; SD, standard deviation

Figure 2. Forest plot olanzapine (n=8)

Figure 3. Forest plot (a) olanzapine study (n=7) (b) prospective olanzapine study (n=3) (c) retrospective olanzapine study (n=4)

Figure 4. Forest plot clozapine (n=4)

Figure 1

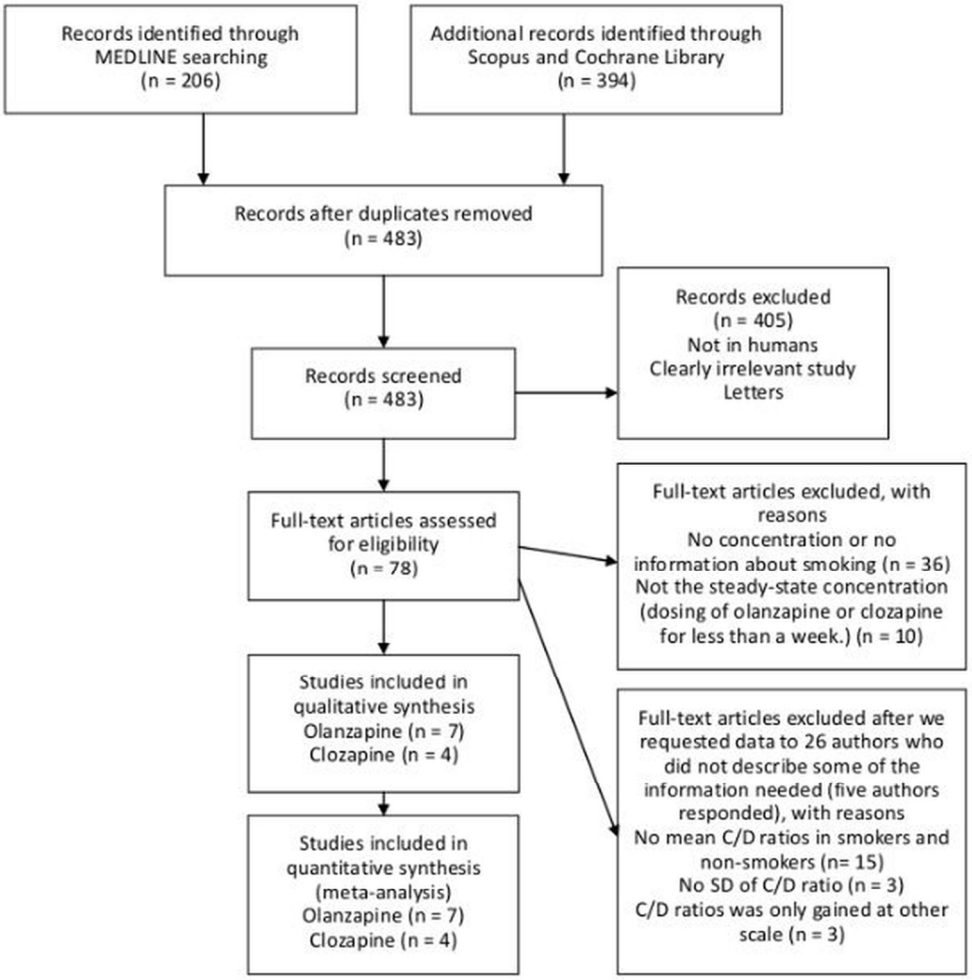

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Figure 2

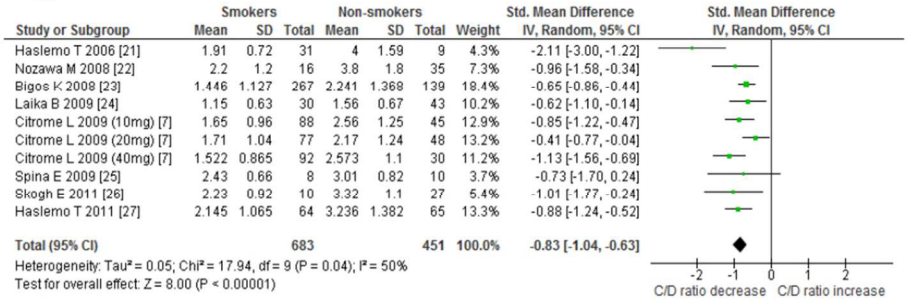

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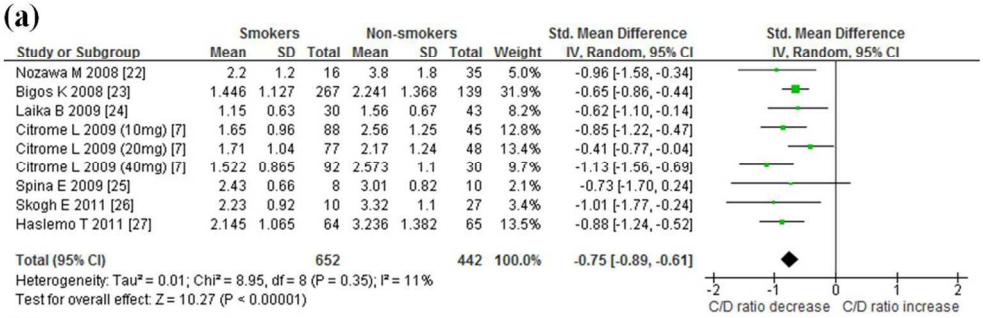
Figure 3

(a)



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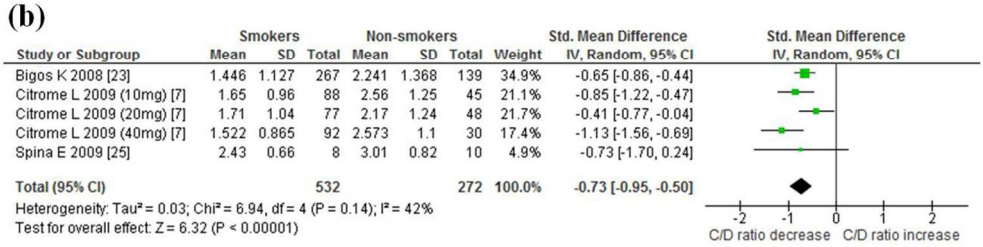

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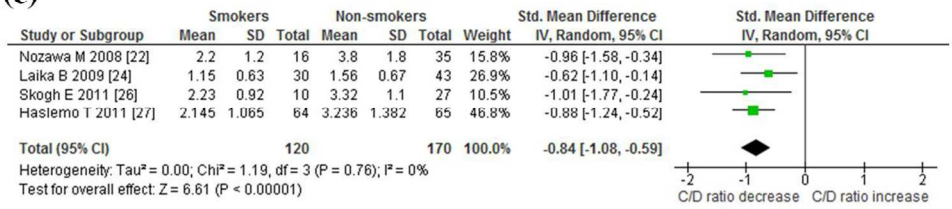

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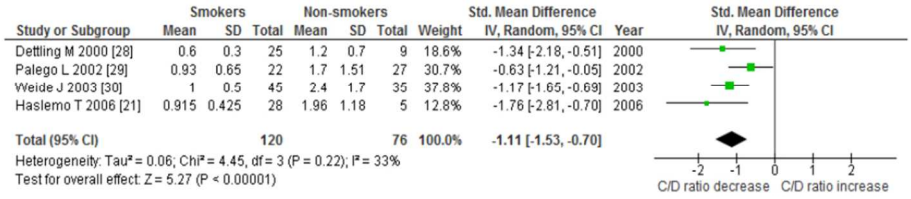
Figure 4



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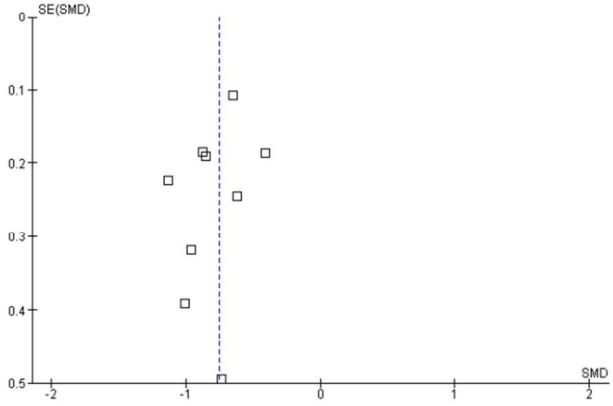
Supplementary figure 1



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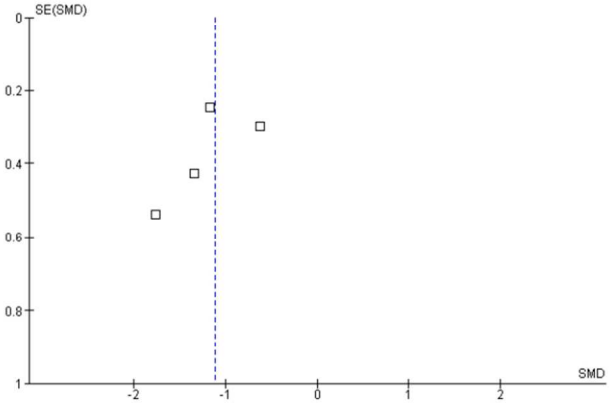
Supplementary figure 2



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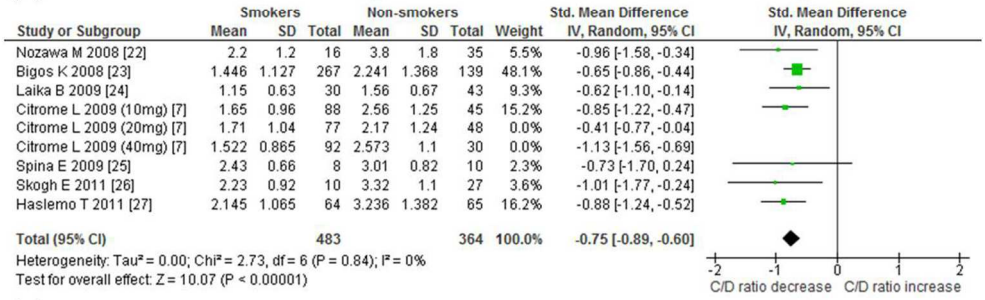
Supplementary figure 3

(a)



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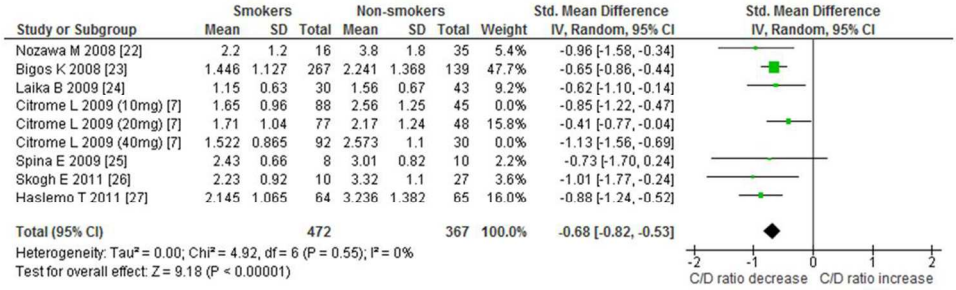

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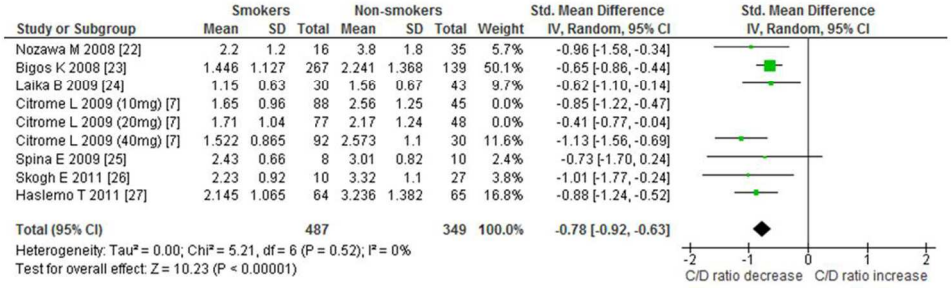

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MOOSE Checklist

Article details:

Title: Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

Authors: Yoshiyuki Tsuda, Junji Saruwatari, Norio Yasui-Furukori

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of two commonly used antipsychotics, olanzapine and clozapine. However, no definitive agreement regarding the dose adjustment in clinical practice based on the patient's smoking status has been reached.
√	Hypothesis statement	It may be able to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on the smoking status of the patient by conducting a meta-analysis.
√	Description of study outcomes	The mean concentration to dose (C/D) ratio (ng/ml)/(mg/day) of olanzapine and clozapine
√	Type of exposure or intervention used	Olanzapine or clozapine treatment
√	Type of study designs used	We included both prospective and retrospective studies.
√	Study population	The patients with schizophrenia or other psychiatric diseases who were treated with olanzapine or clozapine
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the investigators, Junji Saruwatari and Norio Yasui-Furukori are included in the author list.
√	Search strategy, including time period included in the synthesis and keywords	MEDLINE from 1946 – August 2012 Six terms in which either 'olanzapine' or 'clozapine' was paired with 'smoking' or 'cigarette' or 'tobacco' or 'smoke'.
√	Databases and registries searched	MEDLINE, Scopus and the Cochrane Library
√	Search software used, name and version, including special features	We did not employ any search software.
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for additional references.

√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in Figure 1. The citation list is available upon request.
√	Method of addressing articles published in languages other than English	This meta-analysis excluded the article published in languages other than English.
√	Method of handling abstracts and unpublished studies	We did not search unpublished study.
√	Description of any contact with authors	We requested data from the authors if either the C/D ratio of olanzapine or clozapine or the standard deviation (SD) was not described.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies provided mean C/D ratio and the SD values in smokers and non-smokers, respectively.
√	Assessment of confounding	We confirmed that race and sex could be associated with differences in the disposition of olanzapine using a meta-analysis. However, there was insufficient data available to assess the effects of these factors on the clozapine disposition.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	The quality of the included studies is shown in Table 3.
√	Assessment of heterogeneity	Heterogeneity of the studies was explored with I^2 statistics that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	The weighted mean difference of C/D ratios of olanzapine and clozapine between smokers and non-smokers was calculated by DerSimonian-Laird random effects models.
√	Provision of appropriate tables and graphics	Tables 1-3, Figures 1-4, and Supplementary figures 1-3
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figures 2-4
√	Table giving descriptive information for each study included	Tables 1 and 2
√	Results of sensitivity testing	We conducted subgroup analyses of olanzapine. The subgroup analyses (prospective/retrospective studies) also showed results similar to primary

		analyses of olanzapine. In the meta-analyses of clozapine, no subgroup analyses could be conducted because of the small number of patients included in the study.
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates.
Reporting of discussion should include		
√	Quantitative assessment of bias	Publication bias was not shown in both of analyses of olanzapine and clozapine using Egger test and funnel plot. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo et al., 2006) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias.
√	Justification for exclusion	We excluded the studies from subjects who have not received olanzapine or clozapine for at least a week.
√	Assessment of quality of included studies	We discussed quality of included studies in discussion section.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Based on the findings of the present study, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.
√	Generalization of the conclusions	The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and

		1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. The results of this meta-analysis are useful as standards to regulate dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status. However, this meta-analysis could not take the amount of smoking and adherence into consideration so additional research is required to establish administration plan based on smoking status.
√	Guidelines for future research	Future studies are required to investigate the effect of smoking on olanzapine and clozapine dispositions, while also taking the amount of smoking, adherence, and the other patient's characteristics (e.g., sex, race, genetic polymorphisms) into consideration.
√	Disclosure of funding source	This work was supported by grants-in-aid (Nos. 23510348, 24590652 and 25860117) for scientific research from the Japanese Ministry of Education, Science, Sports and Culture. Tobacco industry funding did not support the manuscript.

PRISMA flow chart: Figure 1

BMJ open

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004216.R2
Article Type:	Research
Date Submitted by the Author:	03-Feb-2014
Complete List of Authors:	Tsuda, Yoshiyuki; Graduate School of Pharmaceutical Sciences, Kumamoto University, Division of Pharmacology and Therapeutics Saruwatari, Junji; Graduate School of Pharmaceutical Sciences, Kumamoto University, Division of Pharmacology and Therapeutics Yasui-Furukori, Norio; Hirosaki University School of Medicine, Department of Neuropsychiatry
Primary Subject Heading:	Pharmacology and therapeutics
Secondary Subject Heading:	Smoking and tobacco, Mental health, Evidence based practice
Keywords:	Schizophrenia & psychotic disorders < PSYCHIATRY, Adverse events < THERAPEUTICS, MENTAL HEALTH, Toxicity < THERAPEUTICS

TITLE

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotic agents, olanzapine and clozapine

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KEY WORDS

olanzapine, clozapine, smoking, meta-analysis, schizophrenia, disposition

WORD COUNT

3783 words

ABSTRACT

Objective: To clarify the effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine, and to create standards to adjust the doses of these drugs in clinical practice based on the smoking status.

Design: A meta-analysis was conducted by searching MEDLINE, Scopus and the Cochrane Library for relevant prospective and retrospective studies.

Included Studies: We included the studies that investigated the effects of smoking on the concentration to dose (C/D) ratio of olanzapine or clozapine.

Primary outcome measure: The weighted mean difference was calculated using a DerSimonian-Laird random effects model, along with 95% confidence intervals (CI).

Results: Seven association studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders, were included in the meta-analysis of olanzapine. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Four association studies of clozapine were included in the meta-analysis of clozapine,

comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers.

Conclusions: We suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

299 words

ARTICLE SUMMARY

Article focus

- Many studies related to the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but there has been no definitive agreement regarding the dose adjustment needed in clinical practice based on smoking status.
- The meta-analyses of prospective and retrospective studies were conducted to clarify the effects of smoking on the disposition of olanzapine and clozapine and to create standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

Key messages

- The mean difference in the concentration to dose (C/D) ratios of olanzapine between smokers and non-smokers was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration.
- The mean difference in the C/D ratios of clozapine between smokers and

non-smokers was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.

- The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

Strengths and limitations of this study

- The major strength of this study is that it clarifies the effects of smoking on the olanzapine and clozapine concentrations in a large population and provides standards that can be used to regulate the dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status.
- The major limitations of the present study are that we could not use another search engine, e.g., Embase and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked.

Additionally, this meta-analysis standardized pharmacokinetic parameters to C/D ratios, and therefore, only seven studies for olanzapine and four studies for clozapine could be included.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug approved for the treatment of schizophrenia, mania and for preventing the recurrence of bipolar disorders[1]. Olanzapine is a thienobenzodiazepine derivate, which shows potent antagonism at $D_{1\text{-}4}$ dopaminergic receptors, as well as 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[2]. Olanzapine is extensively metabolized in the liver, mainly via cytochrome P450 (CYP) 1A2, but also via CYP2D6, CYP3A4, flavin-containing monooxygenase (FMO) and via glucuronidation[2]. Among these enzymes, CYP1A2 accounts for approximately 50% to 60% of olanzapine metabolism[2].

Clozapine is the prototype atypical antipsychotic, and it belongs to the chemical class of the dibenzodiazepines[1]. Clozapine has much greater antagonistic activity on D_4 than D_2 dopaminergic receptors. It also shows a potent antagonism of 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[1]. Clozapine has been widely used following its introduction, because it induces relatively few extrapyramidal effects, and it shows therapeutic benefits for patients who have failed to respond to other agents[3]. Clozapine is rapidly absorbed, and undergoes extensive hepatic metabolism[4]. Various lines of evidence indicate that CYP1A2 and CYP3A4 play a significant role in both *N*-oxidation and *N*-demethylation of the

compound, whereas CYP2D6 plays a minor role in *N*-demethylation[1 4].

The prevalence of smoking is two- to three-fold higher in patients with schizophrenia than that in the general population, and about 58-88% of patients with schizophrenia are current smokers[5]. Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of many drugs, including olanzapine and clozapine[6].

Citrome *et al*., 2009[7] (n=380) reported that the olanzapine concentrations were significantly lower in smokers with schizophrenia than in non-smokers. Previous clinical studies with small numbers of patients with schizophrenia reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total score than non-smokers[8 9]. Meanwhile, although the relationship between the clozapine concentration and clinical outcome is controversial[10-12], it was also reported that smokers who were treated with clozapine suffered side effects (i.e. auditory hallucinations, hallucinations, hypersalivation, drowsiness, clonic seizures, convulsions and unconsciousness) after smoking cessation[4 13-16].

Many studies about the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but no definitive agreement regarding the dose

adjustment in clinical practice based on the patient's smoking status has been reached. There are several reasons for the slow progress in making the smoking-associated dosage selection; (i) the sample sizes of the previous studies were small; (ii) each study used different pharmacokinetic (PK) parameters [e.g., plasma concentration, plasma concentration to dose (C/D) ratio, clearance (CL)] and the degree of the effect of smoking on the dispositions of olanzapine or clozapine was different between studies. Therefore, a meta-analysis has been needed to overcome the limitations of the previous studies and to determine the degree of the effects of smoking on the disposition of olanzapine and clozapine, in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on smoking status of the patient.

In this study, we performed a meta-analysis of the effects of smoking on the disposition of olanzapine and clozapine.

METHODS

Study selection

A preliminary search of the literature covering the period from 1946 to August 2012 was undertaken to identify publications related to the effects of smoking on the

disposition of olanzapine and clozapine. Electronic databases, including MEDLINE, Scopus and the Cochrane Library, were initially searched using six terms, in which either ‘olanzapine’ or ‘clozapine’ was paired with ‘smoking’ or ‘cigarette’ or ‘tobacco’ or ‘smoke’. We excluded other than English publications, and studies not performed on human participants, after the search. The inclusion criteria were as follows: (i) published in a peer-reviewed journal; (ii) contained the mean C/D ratios (ng/mL)/(mg/day) of olanzapine or clozapine, and their standard deviation (SD) in smokers and non-smokers, respectively, and we requested data from the author(s) if either the mean C/D ratios or the SD was not described; and (iii) the data were from subjects who had received olanzapine or clozapine for at least a week. In this study, the smokers were defined as the subjects who were currently smoking. Additionally, we divided the selected publications into two groups, i.e. olanzapine and clozapine study groups (Figure 1).

The review and analysis were conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement as a guide[17]. Two researchers (YT and JS) independently searched the literature. Once all the papers had been assessed, any discrepancies in the answers were identified and discussed between the scorers to reach a consensus on the single best option. Any points of assessment on which the scorers could not reach an agreement were resolved by a third scorer (Y-FN).

The data were extracted from each article using a standardized form including the first author, publication year and other information, as described in the following section.

Data extraction

The number of patients, the mean values of the C/D ratios and the SD values of these ratios were extracted for smokers and non-smokers, respectively, from the selected publications. The C/D ratios were standardised to be in the same units, i.e. (ng/mL)/(mg/day). When the values were not described or they were drawn on other scale [e.g., (ng/mL)/(mg/kg)], we asked the author(s) to send us their data in the desired units. We tried to gather information by requesting it from 26 authors. Although five authors responded to our requests, the other 21 studies of olanzapine or clozapine could not be included due to a lack of information (the mean C/D ratios and SD were not available for smokers and non-smokers, respectively, from 14 studies, the SD was not given in four studies, and the mean C/D ratios was described on other scale, i.e. (ng/ml)(mg/kg), in three studies) (Figure 1).

The characteristics of the studies included in this meta-analysis of the effects of smoking on the disposition of olanzapine or clozapine are shown in Tables 1 and 2. We systematically assessed several key points of study quality proposed by the MOOSE

Collaboration[18]. The quality of the included studies is shown in Table 3.

Table 1. The characteristics of the included olanzapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Haslemo T *et al*., 2006	Norway	Retrospective study	40 (31)	29/11	Schizophrenia	Unknown	40 – 71
Nozawa M *et al*., 2008	Japan	Retrospective study	51 (16)	34/17	Schizophrenia	DSM-IV	32.6 ± 9.6
Bigos KL *et al*., 2008	USA	Prospective study	406 (267)	289/117	Schizophrenia	DSM-IV	42 ± 7.9
Laika B *et al*., 2009	Germany	Retrospective study	73 (30)	36/37	Schizophrenia, Mood disorder	ICD-10	41.7 ± 14.7
Citrome L *et al*., 2009	USA	Prospective study	380 (257)	265/115	Schizophrenia, Schizoaffective	DSM-IV	18 – 60

					disorder		
					Bipolar disorder,		
Spina E *et al*., 2009	Italy	Prospective study	18 (8)	10/8	Schizoaffective	DSM-IV	39.3 ± 8.6
					disorder		
					Schizophrenia,		
Skogh E *et al*., 2011	Sweden	Retrospective study	37 (10)	25/12	Schizoaffective	DSM-IV	23 – 50
					disorder		
Haslemo T *et al*., 2011	Norway	Retrospective study	129 (64)	0/129	Unknown	Unknown	18 – 40

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.

Table 2. The characteristics of the included clozapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Dettling M *et al*., 2000	Germany	Retrospective study	34 (25)	18/16	Schizophrenia, Bipolar disorder	DSM-III-R	33.7 ± 10.6
Palego L *et al*., 2002	USA	Retrospective study	49 (22)	25/24	Schizophrenia, Schizoaffective disorder	DSM-IV	36.84 ± 1.96 (SE)
Weide J *et al*., 2003	Netherlands	Retrospective study	80 (45)	51/29	Schizophrenia	Unknown	18 – 86
Haslemo T *et al*., 2006	Norway	Retrospective study	33 (28)	21/12	Schizophrenia	Unknown	28 – 62

DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Third Edition-Revised; DSM-IV, Diagnostic and Statistical Manual

of Mental Disorders Fourth Edition.

Table 3. The quality of the included studies

First author	Publication year	Drug treatment	Number of smokers	Diagnostic criteria	Treatment duration	Measurement of blood drug concentration	Sampling scheme	Total score
Haslemo T	2006	Olanzapine	Yes	NA	Yes	Yes	Yes	4
Nozawa M	2008	Olanzapine	Yes	Yes	Yes	Yes	NA	4
Bigos KL	2008	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Laika B	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Citrome L	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Spina E	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Skogh E	2011	Olanzapine	Yes	Yes	Yes	Yes	Yes	5

Haslemo T	2011	Olanzapine	Yes	NA	NA	Yes	Yes	3
Dettling M	2000	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Palego L	2002	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Weide J	2003	Clozapine	Yes	NA	Yes	Yes	Yes	4
Haslemo T	2006	Clozapine	Yes	NA	Yes	Yes	Yes	4

NA, not available.

Statistical analysis

A meta-analysis using the weighted mean difference in the C/D ratios of olanzapine or clozapine between smokers and non-smokers was performed using the Review Manager (RevMan) Version 5.1 for Windows software program (Cochrane Collaboration, http://www.cc-ims.net/RevMan). Cochran's chi-square-based Q-statistic test was applied to assess the between-study heterogeneity. The weighted mean difference was calculated using DerSimonian-Laird random effects models[19], along with 95% confidence intervals (CI), to measure the strength of the association. In this study, we applied the random effects model for the comparisons, which is more conservative because of the possibility that the underlying effect differed across studies and populations. The weighted mean difference was also calculated when the studies were stratified according to the study design, i.e. prospective or retrospective study. We used the I^2 statistic to assess the heterogeneity of the results. Publication bias was assessed by visually examining a funnel plot with asymmetry and formally assessing publication bias with the Egger test[20]. The statistical significance level for all analyses was set at a two-sided value of $p<0.05$.

RESULTS

Olanzapine: Search results and study characteristics

Eight studies of olanzapine[7 21-27] met our criteria (Figure 1). The studies included in this analysis for olanzapine are listed in Table 1. Since the study by Citrome *et al*., 2009[7] was derived from a randomized clinical trial of 10, 20, and 40 mg as the daily olanzapine dose in patients with schizophrenia or schizoaffective disorder, we divided its populations into three groups according to the respective olanzapine doses. Since the study by Spina *et al*., 2009[25] focused on the effects of valproate on the olanzapine plasma concentrations, so we extracted the C/D ratios of olanzapine at baseline (before taking valproate). The study by Haslemo *et al*., 2011[27] focused on the effects of contraceptives on the serum concentration of olanzapine among female patients who were treated either with olanzapine alone or the combination of estradiol-containing contraceptives, so we requested the C/D ratios in subjects not using any contraceptives that can affect the CYP1A2 activity.

Primary analyses of olanzapine

The weighted mean difference was derived from all studies, comprising a total of 1134 patients (683 smokers and 451 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in

non-smokers (p<0.00001) (Figure 2), and the mean difference was -0.83 (ng/mL)/(mg/day) (95% CI: -1.04 to -0.63). Although there was no significant publication bias (p=0.26), significant heterogeneity was observed (I^2=50, p=0.04). Since we included two studies by the same authors, we excluded the older study (Haslemo *et al*., 2006[21]) in the subsequent analyses to reduce the heterogeneity.

The analysis from the seven studies showed that there was no significant heterogeneity among the mean differences (I^2=11%, p= 0.35) (Figure 3a). The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 3a), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI: -0.89 to -0.61). No significant publication bias was shown using the Egger test in the studies of olanzapine (p=0.282). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 1).

Subgroup analyses of olanzapine

Prospective studies

We conducted subgroup analyses to confirm the precision of the primary

analyses. Of the seven included studies of olanzapine, three were prospective studies, while four were retrospective studies. In the prospective studies (532 smokers and 272 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3b), and the mean difference was -0.73 (ng/mL)/(mg/day) (95% CI: -0.95 to -0.50).

Retrospective studies

In the retrospective studies (120 smokers and 170 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3c), and the mean difference was -0.84 (ng/mL)/(mg/day) (95% CI: -1.08 to -0.59).

Clozapine: Search results and study characteristics

Four studies regarding the clozapine disposition[21 28-30] met our criteria, all of which were retrospective studies (Figure 1). The clozapine studies included in this analysis are listed in Table 2.

Analyses of clozapine

There was no significant heterogeneity among the mean differences (I^2=33%,

p=0.22) (Figure 4). The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 4), and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). No significant bias was shown using the Egger test for the clozapine studies (p=0.436). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 2).

DISCUSSION

Smoking is a well-known cause of significant drug interactions in humans[31-33]. The polyaromatic hydrocarbons in cigarette smoke are known to induce CYP1A2[34], and therefore, cigarette smoking can affect the disposition of drugs that are metabolized by CYP1A2, such as olanzapine and clozapine. The prevalence of current smokers is higher in patients with schizophrenia than that in the general population[5]. However, at present, there is no definitive data regarding the dose adjustments of olanzapine and clozapine in clinical practice based on the patient's smoking status. This is the first meta-analysis to clarify the effects of smoking on the disposition of these drugs.

Olanzapine

In the meta-analysis of olanzapine, 1094 patients (652 smokers and 442 non-smokers) from seven clinical studies of olanzapine were evaluated. The results showed that the C/D ratio of olanzapine was 0.75 (ng/mL)/(mg/day) lower in smokers than in non-smokers. The subgroup analyses (prospective/retrospective studies) also showed similar results. Approximately 85% of the oral olanzapine dose is absorbed, but as about 40% is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60%[1]. The mean half-life, mean apparent drug plasma CL and mean apparent volume of distribution of olanzapine were 33 hours, 26 L/h and 1150 L in healthy individuals[35]. Previous clinical studies demonstrated that the C/D ratio of olanzapine significantly correlated with a decrease in the Brief Psychiatric Rating Scale[8 9]. The association between the clinical outcome and the plasma olanzapine concentration is clearly curvilinear, with clinical efficacy being approximately associated with a plasma olanzapine concentration range of 20-50 ng/mL[1]. Bigos *et al*., 2008[23] (n=523) analyzed the population pharmacokinetics of olanzapine, and they determined that sex, smoking and race contribute to the variability in olanzapine clearance. The study also demonstrated that smoking increased the olanzapine clearance by 55%, while also incorporating other confounding factors. Based on the findings of the present study, it

was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 30% in non-smokers compared with smokers.

Clozapine

In the meta-analysis of clozapine, 196 patients (smokers: 120, non-smokers: 76) from four clinical studies were evaluated. The results showed that the C/D ratio of clozapine was 1.11 (ng/mL)/(mg/day) lower in smokers than in non-smokers. After oral administration of clozapine, the drug is rapidly absorbed. Only 27-50% of the dose reaches the systemic circulation unchanged, because of extensive first-pass metabolism[1]. There is a wide inter-patitent variability in PK parameters of clozapine[1]. The mean half-life of clozapine ranges from 9 to 17 hours[1]. The plasma CL of clozapine was reported to be between 9 and 53 L/hour, and the volume of distribution of clozapine was between 2 and 7 L/kg[1]. The steady-state plasma concentrations of clozapine are reached after 7-10 days of dosing[1]. The relationship between the clozapine concentration and clinical outcome is controversial. According to the study by Spina *et al.*, 2000[11], a

receiver operating characteristics analysis showed that a clozapine concentration cut-off value of 350 ng/mL distinguished responders and non-responders with a sensitivity of 72% and a specificity of 70%. On the other hand, it has been suggested that the clozapine concentration does not correlate with the decrease in the Brief Psychiatric Rating Scale[10 12].

A recent review summarized the previous studies regarding the relationships between the clozapine concentrations and clinical response, and suggested that clozapine levels above 250-400 ng/mL are associated with an increased chance of a clinical response [36]. Moreover, clozapine doses exceeding 500-600 mg/day of clozapine could carry an increased risk of seizures[36]. Because the smokers who were treated with clozapine were reported to suffer serious central nervous side effects after smoking cessation[4 13-16], it is necessary to regulate the clozapine dosage carefully when smokers stop smoking or decrease the amount of smoking. Li *et al*., 2012[36] applied nonlinear mixed-effect modelling to characterize the pharmacokinetics of clozapine in Chinese patients. In the final model, sex and the smoking status were identified as significant covariates for the clearance of clozapine and norclozapine[36], and smokers had a 1.45-fold higher clearance of clozapine than non-smokers[36]. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual

doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of clozapine should be reduced by 50% in non-smokers compared with smokers.

Other factors affecting the disposition of olanzapine and clozapine

Many previous clinical studies reported that sex, race, age, co-medication and the genotype could affect the disposition of olanzapine and clozapine[23 37-47]. Since estrogen is known to inhibit the activity of CYP1A2[23], it is not surprising that the clearance of olanzapine and clozapine was reported to be lower in females than in males[23]. Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as fluoxetine and fluvoxamine, were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4[27 41 43 45 48]. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4[41 45 48 49]. Race is known to be associated with variability in the CYP1A2 activity. Bigos *et al*., 2008[23] reported that African Americans cleared

olanzapine faster than did other races (i.e., Caucasians, Asians and Native Americans). Moreover, many genetic polymorphisms were reported to affect to the disposition of olanzapine and clozapine. A recent review suggested that *UGT1A4*3*, *CYP1A2* rs2472297, *FMO3* K158-G308, *FMO1*6*, *FMO1* rs7877 and *CYP3A43* rs472660 polymorphisms all influence the olanzapine metabolism[50]. Regarding clozapine, Lee *et al*., 2012[44] showed that *CYP1A2* rs2069521 and rs2069522 polymorphisms were significantly associated with the C/D ratio of clozapine, and *CYP2D6* rs1135840 was associated with the ratio of norclozapine and clozapine. Nevertheless, in the present study, there was insufficient data available to assess the effects of these factors on the disposition of olanzapine or clozapine. Moreover, the influence of smoking on the disposition of olanzapine and clozapine might be different among different patient populations (e.g., the elderly, females, different diagnostic groups), but we could not conduct a meta-analysis for these populations.

Strengths and limitations of the study

The major strengths of this study are that it synthesized the previous studies with standardization of the PK parameters to the C/D ratios, that it clarified the degree of the effect of smoking on the C/D ratios and that it provided standards that can be

used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

On the other hand, there are several limitations to this meta-analysis. The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked. This meta-analysis standardized the PK parameters to C/D ratios (ng/mL)/(mg/day), and therefore, only seven studies for olanzapine and four studies for clozapine could be included. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for

smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo *et al*., 2006[21]) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias. Furthermore, we included the three results from Citrome *et a*l., 2009[7] independently, and therefore, should verify the correlation of these results using a random intercept in the mixed effects meta-analysis. When the three results were separately included in the meta-analysis, the weighted differences were not significantly different among the analyses (Supplementary figure 3). However, we could not apply the random intercept in the mixed effects meta-analysis, because we used the Review Manager (RevMan) software program, which lacks this function for the analysis. In previous studies, the sum concentrations of clozapine and its metabolite, norclozapine, and the norclozapine to clozapine ratio, were also used as a clinical outcome and an index of metabolic activity, respectively[1]. However, we could not use these parameters for the present meta-analysis, because we used only the clozapine concentration to dose ratio in order to be able to include as many studies as possible and to develop simple standards that can be used in clinical practice.

The other limitation is that this meta-analysis simply divided subjects into

smokers and non-smokers, so the amount of smoking was not able to be taken into consideration. It has been suggested that the smoking-induced changes in hepatic CYP1A2 abundance are dependent on the daily cigarette consumption[51]. Therefore, the differences in the amounts of smoking might have contributed to the variations in the influence of cigarette smoking on the disposition of olanzapine and clozapine among the studies included. Additionally, this meta-analysis could not confirm patient adherence. It was previously reported that up to 80 % of patients with schizophrenia are at least partially nonadherent[52], and this might have affected the results. Although we included the studies that described that the subjects had taken the drug for at least a week, we could not obtain any information regarding the adherence, because none of the studies clearly described this information. Finally, the use of co-medications, which may affect the disposition of olanzapine or clozapine, could not be excluded. Six subjects in the study by Laika *et al*., 2010[24] were taking carbamazepine and 21 subjects in the study by Weide *et al*., 2003 were taking carbamazepine or fluvoxamine. These drugs are known to affect the activity of CYP1A2 and/or CYP3A4, which is also involved in the metabolism of olanzapine and clozapine.

CONCLUSION

This meta-analysis synthesized previous studies and represented the effects of smoking on the disposition of olanzapine and clozapine in a way that can be used to change the current clinical practices. Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

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Contributors

YT reviewed all the abstracts, reviewed all the full papers, performed the statistical analysis and wrote the paper. JS and NY-F reviewed all of the abstracts and full papers for relevance, and wrote and reviewed the submitted article.

Competing interests

We declare no competing interests.

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Data sharing statement

There are no additional data available.

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Figure Legends

Figure 1. A flow chart of the study selection process

Abbreviations: C/D, concentration to dose; SD, standard deviation

Figure 2. Forest plot olanzapine (n=8)

Figure 3. Forest plot (a) olanzapine study (n=7) (b) prospective olanzapine study (n=3) (c) retrospective olanzapine study (n=4)

Figure 4. Forest plot clozapine (n=4)

Supplementary Figure legends

Supplementary Figure 1. The funnel plot of olanzapine (n=7) (the study by Citrome *et al*., 2009 is represented by three data points in this figure)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 2. The funnel plot of clozapine (n=4)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 3. The forest plot of olanzapine (n=7) (a) including only the data for 10 mg olanzapine reported by Citrome *et al*., 2009, (b) including only the data for 20 mg olanzapine reported by Citrome *et al*., 2009 and (c) including only the data for 40 mg olanzapine reported by Citrome *et al*., 2009

TITLE

Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotic agents, olanzapine and clozapine

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ABSTRACT

Objective: To clarify the effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine, and to create standards to adjust the doses of these drugs in clinical practice based on the smoking status.

Design: A meta-analysis was conducted by searching MEDLINE, Scopus and the Cochrane Library for relevant prospective and retrospective studies.

Included Studies: We included the studies that investigated the effects of smoking on the concentration to dose (C/D) ratio of olanzapine or clozapine.

Primary outcome measure: The weighted mean difference was calculated using a DerSimonian-Laird random effects model, along with 95% confidence intervals (CI).

Results: Seven association studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders, were included in the meta-analysis of olanzapine. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Four association studies of clozapine were included in the meta-analysis of clozapine,

comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p < 0.00001$) and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers.

Conclusions: We suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

299 words

ARTICLE SUMMARY

Article focus

- Many studies related to the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but there has been no definitive agreement regarding the dose adjustment needed in clinical practice based on smoking status.
- The meta-analyses of prospective and retrospective studies were conducted to clarify the effects of smoking on the disposition of olanzapine and clozapine and to create standards that can be used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

Key messages

- The mean difference in the concentration to dose (C/D) ratios of olanzapine between smokers and non-smokers was -0.75 (ng/mL)/(mg/day) (95% CI -0.89 to -0.61). Therefore, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration.
- The mean difference in the C/D ratios of clozapine between smokers and

non-smokers was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). Therefore, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.

- The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration.

Strengths and limitations of this study

- The major strength of this study is that it clarifies the effects of smoking on the olanzapine and clozapine concentrations in a large population and provides standards that can be used to regulate the dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status.
- The major limitations of the present study are that we could not use another search engine, e.g., Embase and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked.

Additionally, this meta-analysis standardized pharmacokinetic parameters to C/D ratios, and therefore, only seven studies for olanzapine and four studies for clozapine could be included.

INTRODUCTION

Olanzapine is an atypical antipsychotic drug approved for the treatment of schizophrenia, mania and for preventing the recurrence of bipolar disorders[1]. Olanzapine is a thienobenzodiazepine derivate, which shows potent antagonism at $D_{1\text{-}4}$ dopaminergic receptors, as well as 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[2]. Olanzapine is extensively metabolized in the liver, mainly via cytochrome P450 (CYP) 1A2, but also via CYP2D6, CYP3A4, flavin-containing monooxygenase (FMO) and via glucuronidation[2]. Among these enzymes, CYP1A2 accounts for approximately 50% to 60% of olanzapine metabolism[2].

Clozapine is the prototype atypical antipsychotic, and it belongs to the chemical class of the dibenzodiazepines[1]. Clozapine has much greater antagonistic activity on D_4 than D_2 dopaminergic receptors. It also shows a potent antagonism of 5-HT_{2A} and 5-HT_{2C} serotonergic, α_1-adrenergic, muscarinic and H_1 histamine receptors[1]. Clozapine has been widely used following its introduction, because it induces relatively few extrapyramidal effects, and it shows therapeutic benefits for patients who have failed to respond to other agents[3]. Clozapine is rapidly absorbed, and undergoes extensive hepatic metabolism[4]. Various lines of evidence indicate that CYP1A2 and CYP3A4 play a significant role in both *N*-oxidation and *N*-demethylation of the

compound, whereas CYP2D6 plays a minor role in *N*-demethylation[1 4].

The prevalence of smoking is two- to three-fold higher in patients with schizophrenia than that in the general population, and about 58-88% of patients with schizophrenia are current smokers[5]. Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of many drugs, including olanzapine and clozapine[6].

Citrome *et al*., 2009[7] (n=380) reported that the olanzapine concentrations were significantly lower in smokers with schizophrenia than in non-smokers. Previous clinical studies with small numbers of patients with schizophrenia reported that smokers had an approximately five-fold lower dose-corrected steady-state plasma olanzapine concentration and a lower decrease in the Brief Psychiatric Rating Scale-total score than non-smokers[8 9]. Meanwhile, although the relationship between the clozapine concentration and clinical outcome is controversial[10-12], it was also reported that smokers who were treated with clozapine suffered side effects (i.e. auditory hallucinations, hallucinations, hypersalivation, drowsiness, clonic seizures, convulsions and unconsciousness) after smoking cessation[4 13-16].

Many studies about the effects of smoking on the disposition of olanzapine and clozapine have been undertaken, but no definitive agreement regarding the dose

adjustment in clinical practice based on the patient's smoking status has been reached. There are several reasons for the slow progress in making the smoking-associated dosage selection; (i) the sample sizes of the previous studies were small; (ii) each study used different pharmacokinetic (PK) parameters [e.g., plasma concentration, plasma concentration to dose (C/D) ratio, clearance (CL)] and the degree of the effect of smoking on the dispositions of olanzapine or clozapine was different between studies. Therefore, a meta-analysis has been needed to overcome the limitations of the previous studies and to determine the degree of the effects of smoking on the disposition of olanzapine and clozapine, in order to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on smoking status of the patient.

In this study, we performed a meta-analysis of the effects of smoking on the disposition of olanzapine and clozapine.

METHODS

Study selection

A preliminary search of the literature covering the period from 1946 to August 2012 was undertaken to identify publications related to the effects of smoking on the

disposition of olanzapine and clozapine. Electronic databases, including MEDLINE, Scopus and the Cochrane Library, were initially searched using six terms, in which either ‘olanzapine’ or ‘clozapine’ was paired with ‘smoking’ or ‘cigarette’ or ‘tobacco’ or ‘smoke’. We excluded other than English publications, and studies not performed on human participants, after the search. The inclusion criteria were as follows: (i) published in a peer-reviewed journal; (ii) contained the mean C/D ratios (ng/mL)/(mg/day) of olanzapine or clozapine, and their standard deviation (SD) in smokers and non-smokers, respectively, and we requested data from the author(s) if either the mean C/D ratios or the SD was not described; and (iii) the data were from subjects who had received olanzapine or clozapine for at least a week. In this study, the smokers were defined as the subjects who were currently smoking. Additionally, we divided the selected publications into two groups, i.e. olanzapine and clozapine study groups (Figure 1).

The review and analysis were conducted using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement as a guide[17]. Two researchers (YT and JS) independently searched the literature. Once all the papers had been assessed, any discrepancies in the answers were identified and discussed between the scorers to reach a consensus on the single best option. Any points of assessment on which the scorers could not reach an agreement were resolved by a third scorer (Y-FN).

The data were extracted from each article using a standardized form including the first author, publication year and other information, as described in the following section.

Data extraction

The number of patients, the mean values of the C/D ratios and the SD values of these ratios were extracted for smokers and non-smokers, respectively, from the selected publications. The C/D ratios were standardised to be in the same units, i.e. (ng/mL)/(mg/day). When the values were not described or they were drawn on other scale [e.g., (ng/mL)/(mg/kg)], we asked the author(s) to send us their data in the desired units. We tried to gather information by requesting it from 26 authors. Although five authors responded to our requests, the other 21 studies of olanzapine or clozapine could not be included due to a lack of information (the mean C/D ratios and SD were not available for smokers and non-smokers, respectively, from 14 studies, the SD was not given in four studies, and the mean C/D ratios was described on other scale, i.e. (ng/ml)(mg/kg), in three studies) (Figure 1).

The characteristics of the studies included in this meta-analysis of the effects of smoking on the disposition of olanzapine or clozapine are shown in Tables 1 and 2. We systematically assessed several key points of study quality proposed by the MOOSE

Collaboration[18]. The quality of the included studies is shown in Table 3.

Table 1. The characteristics of the included olanzapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Haslemo T *et al*., 2006	Norway	Retrospective study	40 (31)	29/11	Schizophrenia	Unknown	40 – 71
Nozawa M *et al*., 2008	Japan	Retrospective study	51 (16)	34/17	Schizophrenia	DSM-IV	32.6 ± 9.6
Bigos KL *et al*., 2008	USA	Prospective study	406 (267)	289/117	Schizophrenia	DSM-IV	42 ± 7.9
Laika B *et al*., 2009	Germany	Retrospective study	73 (30)	36/37	Schizophrenia, Mood disorder	ICD-10	41.7 ± 14.7
Citrome L *et al*., 2009	USA	Prospective study	380 (257)	265/115	Schizophrenia, Schizoaffective	DSM-IV	18 – 60

					disorder		
					Bipolar disorder,		
Spina E *et al*., 2009	Italy	Prospective study	18 (8)	10/8	Schizoaffective	DSM-IV	39.3 ± 8.6
					disorder		
					Schizophrenia,		
Skogh E *et al*., 2011	Sweden	Retrospective study	37 (10)	25/12	Schizoaffective	DSM-IV	23 – 50
					disorder		
Haslemo T *et al*., 2011	Norway	Retrospective study	129 (64)	0/129	Unknown	Unknown	18 – 40

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders Fourth Edition; ICD-10, International Statistical Classification of Diseases and Related Health Problems 10th Revision.

Table 2. The characteristics of the included clozapine studies

Study	Country	Study design	Number of subjects (smokers)	Gender (male/female)	Disease	Diagnosis	Age (mean ± SD or range)
Dettling M *et al*., 2000	Germany	Retrospective study	34 (25)	18/16	Schizophrenia, Bipolar disorder	DSM-III-R	33.7 ± 10.6
Palego L *et al*., 2002	USA	Retrospective study	49 (22)	25/24	Schizophrenia, Schizoaffective disorder	DSM-IV	36.84 ± 1.96 (SE)
Weide J *et al*., 2003	Netherlands	Retrospective study	80 (45)	51/29	Schizophrenia	Unknown	18 – 86
Haslemo T *et al*., 2006	Norway	Retrospective study	33 (28)	21/12	Schizophrenia	Unknown	28 – 62

DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders Third Edition-Revised; DSM-IV, Diagnostic and Statistical Manual

of Mental Disorders Fourth Edition.

Table 3. The quality of the included studies

First author	Publication year	Drug treatment	Number of smokers	Diagnostic criteria	Treatment duration	Measurement of blood drug concentration	Sampling scheme	Total score
Haslemo T	2006	Olanzapine	Yes	NA	Yes	Yes	Yes	4
Nozawa M	2008	Olanzapine	Yes	Yes	Yes	Yes	NA	4
Bigos KL	2008	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Laika B	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Citrome L	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Spina E	2009	Olanzapine	Yes	Yes	Yes	Yes	Yes	5
Skogh E	2011	Olanzapine	Yes	Yes	Yes	Yes	Yes	5

Haslemo T	2011	Olanzapine	Yes	NA	NA	Yes	Yes	3
Dettling M	2000	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Palego L	2002	Clozapine	Yes	Yes	Yes	Yes	Yes	5
Weide J	2003	Clozapine	Yes	NA	Yes	Yes	Yes	4
Haslemo T	2006	Clozapine	Yes	NA	Yes	Yes	Yes	4

NA, not available.

Statistical analysis

A meta-analysis using the weighted mean difference in the C/D ratios of olanzapine or clozapine between smokers and non-smokers was performed using the Review Manager (RevMan) Version 5.1 for Windows software program (Cochrane Collaboration, http://www.cc-ims.net/RevMan). Cochran's chi-square-based Q-statistic test was applied to assess the between-study heterogeneity. The weighted mean difference was calculated using DerSimonian-Laird random effects models[19], along with 95% confidence intervals (CI), to measure the strength of the association. In this study, we applied the random effects model for the comparisons, which is more conservative because of the possibility that the underlying effect differed across studies and populations. The weighted mean difference was also calculated when the studies were stratified according to the study design, i.e. prospective or retrospective study. We used the I^2 statistic to assess the heterogeneity of the results. Publication bias was assessed by visually examining a funnel plot with asymmetry and formally assessing publication bias with the Egger test[20]. The statistical significance level for all analyses was set at a two-sided value of $p<0.05$.

RESULTS

Olanzapine: Search results and study characteristics

Eight studies of olanzapine[7 21-27] met our criteria (Figure 1). The studies included in this analysis for olanzapine are listed in Table 1. Since the study by Citrome *et al*., 2009[7] was derived from a randomized clinical trial of 10, 20, and 40 mg as the daily olanzapine dose in patients with schizophrenia or schizoaffective disorder, we divided its populations into three groups according to the respective olanzapine doses. Since the study by Spina *et al*., 2009[25] focused on the effects of valproate on the olanzapine plasma concentrations, so we extracted the C/D ratios of olanzapine at baseline (before taking valproate). The study by Haslemo *et al*., 2011[27] focused on the effects of contraceptives on the serum concentration of olanzapine among female patients who were treated either with olanzapine alone or the combination of estradiol-containing contraceptives, so we requested the C/D ratios in subjects not using any contraceptives that can affect the CYP1A2 activity.

Primary analyses of olanzapine

The weighted mean difference was derived from all studies, comprising a total of 1134 patients (683 smokers and 451 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in

non-smokers (p<0.00001) (Figure 2), and the mean difference was -0.83 (ng/mL)/(mg/day) (95% CI: -1.04 to -0.63). Although there was no significant publication bias (p=0.26), significant heterogeneity was observed (I^2=50, p=0.04). Since we included two studies by the same authors, we excluded the older study (Haslemo *et al*., 2006[21]) in the subsequent analyses to reduce the heterogeneity.

The analysis from the seven studies showed that there was no significant heterogeneity among the mean differences (I^2=11%, p= 0.35) (Figure 3a). The weighted mean difference was derived from all studies, comprising 1094 patients (652 smokers and 442 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers (p<0.00001) (Figure 3a), and the mean difference was -0.75 (ng/mL)/(mg/day) (95% CI: -0.89 to -0.61). No significant publication bias was shown using the Egger test in the studies of olanzapine (p=0.282). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 1).

Subgroup analyses of olanzapine

Prospective studies

We conducted subgroup analyses to confirm the precision of the primary

analyses. Of the seven included studies of olanzapine, three were prospective studies, while four were retrospective studies. In the prospective studies (532 smokers and 272 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3b), and the mean difference was -0.73 (ng/mL)/(mg/day) (95% CI: -0.95 to -0.50).

Retrospective studies

In the retrospective studies (120 smokers and 170 non-smokers), the C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 3c), and the mean difference was -0.84 (ng/mL)/(mg/day) (95% CI: -1.08 to -0.59).

Clozapine: Search results and study characteristics

Four studies regarding the clozapine disposition[21 28-30] met our criteria, all of which were retrospective studies (Figure 1). The clozapine studies included in this analysis are listed in Table 2.

Analyses of clozapine

There was no significant heterogeneity among the mean differences (I^2=33%,

p=0.22) (Figure 4). The weighted mean difference was derived from all studies, comprising 196 patients (120 smokers and 76 non-smokers) with schizophrenia or other psychiatric disorders. The C/D ratio was significantly lower in smokers than in non-smokers ($p<0.00001$) (Figure 4), and the mean difference was -1.11 (ng/mL)/(mg/day) (95% CI -1.53 to -0.70). No significant bias was shown using the Egger test for the clozapine studies (p=0.436). The funnel plot also suggested that publication bias was unlikely (Supplementary figure 2).

DISCUSSION

Smoking is a well-known cause of significant drug interactions in humans[31-33]. The polyaromatic hydrocarbons in cigarette smoke are known to induce CYP1A2[34], and therefore, cigarette smoking can affect the disposition of drugs that are metabolized by CYP1A2, such as olanzapine and clozapine. The prevalence of current smokers is higher in patients with schizophrenia than that in the general population[5]. However, at present, there is no definitive data regarding the dose adjustments of olanzapine and clozapine in clinical practice based on the patient's smoking status. This is the first meta-analysis to clarify the effects of smoking on the disposition of these drugs.

Olanzapine

In the meta-analysis of olanzapine, 1094 patients (652 smokers and 442 non-smokers) from seven clinical studies of olanzapine were evaluated. The results showed that the C/D ratio of olanzapine was 0.75 (ng/mL)/(mg/day) lower in smokers than in non-smokers. The subgroup analyses (prospective/retrospective studies) also showed similar results. Approximately 85% of the oral olanzapine dose is absorbed, but as about 40% is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60%[1]. The mean half-life, mean apparent drug plasma CL and mean apparent volume of distribution of olanzapine were 33 hours, 26 L/h and 1150 L in healthy individuals[35]. Previous clinical studies demonstrated that the C/D ratio of olanzapine significantly correlated with a decrease in the Brief Psychiatric Rating Scale[8 9]. The association between the clinical outcome and the plasma olanzapine concentration is clearly curvilinear, with clinical efficacy being approximately associated with a plasma olanzapine concentration range of 20-50 ng/mL[1]. Bigos *et al*., 2008[23] (n=523) analyzed the population pharmacokinetics of olanzapine, and they determined that sex, smoking and race contribute to the variability in olanzapine clearance. The study also demonstrated that smoking increased the olanzapine clearance by 55%, while also incorporating other confounding factors. Based on the findings of the present study, it

was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentrations. These findings imply that the daily doses of olanzapine should be reduced by 30% in non-smokers compared with smokers.

Clozapine

In the meta-analysis of clozapine, 196 patients (smokers: 120, non-smokers: 76) from four clinical studies were evaluated. The results showed that the C/D ratio of clozapine was 1.11 (ng/mL)/(mg/day) lower in smokers than in non-smokers. After oral administration of clozapine, the drug is rapidly absorbed. Only 27-50% of the dose reaches the systemic circulation unchanged, because of extensive first-pass metabolism[1]. There is a wide inter-patitent variability in PK parameters of clozapine[1]. The mean half-life of clozapine ranges from 9 to 17 hours[1]. The plasma CL of clozapine was reported to be between 9 and 53 L/hour, and the volume of distribution of clozapine was between 2 and 7 L/kg[1]. The steady-state plasma concentrations of clozapine are reached after 7-10 days of dosing[1]. The relationship between the clozapine concentration and clinical outcome is controversial. According to the study by Spina *et al.*, 2000[11], a

receiver operating characteristics analysis showed that a clozapine concentration cut-off value of 350 ng/mL distinguished responders and non-responders with a sensitivity of 72% and a specificity of 70%. On the other hand, it has been suggested that the clozapine concentration does not correlate with the decrease in the Brief Psychiatric Rating Scale[10 12].

A recent review summarized the previous studies regarding the relationships between the clozapine concentrations and clinical response, and suggested that clozapine levels above 250-400 ng/mL are associated with an increased chance of a clinical response [36]. Moreover, clozapine doses exceeding 500-600 mg/day of clozapine could carry an increased risk of seizures[36]. Because the smokers who were treated with clozapine were reported to suffer serious central nervous side effects after smoking cessation[4 13-16], it is necessary to regulate the clozapine dosage carefully when smokers stop smoking or decrease the amount of smoking. Li *et al*., 2012[36] applied nonlinear mixed-effect modelling to characterize the pharmacokinetics of clozapine in Chinese patients. In the final model, sex and the smoking status were identified as significant covariates for the clearance of clozapine and norclozapine[36], and smokers had a 1.45-fold higher clearance of clozapine than non-smokers[36]. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual

doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentrations. These findings imply that the daily doses of clozapine should be reduced by 50% in non-smokers compared with smokers.

Other factors affecting the disposition of olanzapine and clozapine

Many previous clinical studies reported that sex, race, age, co-medication and the genotype could affect the disposition of olanzapine and clozapine[23 37-47]. Since estrogen is known to inhibit the activity of CYP1A2[23], it is not surprising that the clearance of olanzapine and clozapine was reported to be lower in females than in males[23]. Co-medications are also known to affect the disposition of both olanzapine and clozapine. Several drugs, such as fluoxetine and fluvoxamine, were reported to increase the blood concentration of olanzapine and/or clozapine through the inhibition of CYP1A2, CYP2D6, CYP3A4 and/or UDP-glucuronyltransferase 1A4[27 41 43 45 48]. Additionally, carbamazepine, phenobarbital and trimipramine were reported to decrease the blood concentrations of olanzapine and/or clozapine through the induction of CYP1A2 or CYP3A4[41 45 48 49]. Race is known to be associated with variability in the CYP1A2 activity. Bigos *et al*., 2008[23] reported that African Americans cleared

olanzapine faster than did other races (i.e., Caucasians, Asians and Native Americans). Moreover, many genetic polymorphisms were reported to affect to the disposition of olanzapine and clozapine. A recent review suggested that *UGT1A4*3*, *CYP1A2* rs2472297, *FMO3* K158-G308, *FMO1*6*, *FMO1* rs7877 and *CYP3A43* rs472660 polymorphisms all influence the olanzapine metabolism[50]. Regarding clozapine, Lee *et al*., 2012[44] showed that *CYP1A2* rs2069521 and rs2069522 polymorphisms were significantly associated with the C/D ratio of clozapine, and *CYP2D6* rs1135840 was associated with the ratio of norclozapine and clozapine. Nevertheless, in the present study, there was insufficient data available to assess the effects of these factors on the disposition of olanzapine or clozapine. Moreover, the influence of smoking on the disposition of olanzapine and clozapine might be different among different patient populations (e.g., the elderly, females, different diagnostic groups), but we could not conduct a meta-analysis for these populations.

Strengths and limitations of the study

The major strengths of this study are that it synthesized the previous studies with standardization of the PK parameters to the C/D ratios, that it clarified the degree of the effect of smoking on the C/D ratios and that it provided standards that can be

used to adjust the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

On the other hand, there are several limitations to this meta-analysis. The major limitations of the present study are that we could not use another search engine, e.g., Embase, due to lack of the access authority, and we could not include the literature published in other languages (not in English) or the data regarding other confounding factors, such as the age, weight, gender, alcohol consumption and how much the subject smoked. This meta-analysis standardized the PK parameters to C/D ratios (ng/mL)/(mg/day), and therefore, only seven studies for olanzapine and four studies for clozapine could be included. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for

smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo *et al*., 2006[21]) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias. Furthermore, we included the three results from Citrome *et a*l., 2009[7] independently, and therefore, should verify the correlation of these results using a random intercept in the mixed effects meta-analysis. When the three results were separately included in the meta-analysis, the weighted differences were not significantly different among the analyses (Supplementary figure 3). However, we could not apply the random intercept in the mixed effects meta-analysis, because we used the Review Manager (RevMan) software program, which lacks this function for the analysis. In previous studies, the sum concentrations of clozapine and its metabolite, norclozapine, and the norclozapine to clozapine ratio, were also used as a clinical outcome and an index of metabolic activity, respectively[1]. However, we could not use these parameters for the present meta-analysis, because we used only the clozapine concentration to dose ratio in order to be able to include as many studies as possible and to develop simple standards that can be used in clinical practice.

The other limitation is that this meta-analysis simply divided subjects into

smokers and non-smokers, so the amount of smoking was not able to be taken into consideration. It has been suggested that the smoking-induced changes in hepatic CYP1A2 abundance are dependent on the daily cigarette consumption[51]. Therefore, the differences in the amounts of smoking might have contributed to the variations in the influence of cigarette smoking on the disposition of olanzapine and clozapine among the studies included. Additionally, this meta-analysis could not confirm patient adherence. It was previously reported that up to 80 % of patients with schizophrenia are at least partially nonadherent[52], and this might have affected the results. Although we included the studies that described that the subjects had taken the drug for at least a week, we could not obtain any information regarding the adherence, because none of the studies clearly described this information. Finally, the use of co-medications, which may affect the disposition of olanzapine or clozapine, could not be excluded. Six subjects in the study by Laika *et al*., 2010[24] were taking carbamazepine and 21 subjects in the study by Weide *et al*., 2003 were taking carbamazepine or fluvoxamine. These drugs are known to affect the activity of CYP1A2 and/or CYP3A4, which is also involved in the metabolism of olanzapine and clozapine.

CONCLUSION

This meta-analysis synthesized previous studies and represented the effects of smoking on the disposition of olanzapine and clozapine in a way that can be used to change the current clinical practices. Based on the results of this meta-analysis, we suggest that the doses of olanzapine and clozapine should be reduced by 30% and 50%, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. These results are useful as standards to change the doses of olanzapine and clozapine in clinical practice based on the patient's smoking status.

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Contributors

YT reviewed all the abstracts, reviewed all the full papers, performed the statistical analysis and wrote the paper. JS and NY-F reviewed all of the abstracts and full papers for relevance, and wrote and reviewed the submitted article.

Competing interests

We declare no competing interests.

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Data sharing statement

There are no additional data available.

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Figure Legends

Figure 1. A flow chart of the study selection process

Abbreviations: C/D, concentration to dose; SD, standard deviation

Figure 2. Forest plot olanzapine (n=8)

Figure 3. Forest plot (a) olanzapine study (n=7) (b) prospective olanzapine study (n=3)

(c) retrospective olanzapine study (n=4)

Figure 4. Forest plot clozapine (n=4)

Supplementary Figure legends

Supplementary Figure 1. The funnel plot of olanzapine (n=7) (the study by Citrome *et al*., 2009 is represented by three data points in this figure)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 2. The funnel plot of clozapine (n=4)

Abbreviations: SMD, standard mean difference; SE, standard error

Supplementary Figure 3. The forest plot of olanzapine (n=7) (a) including only the data for 10 mg olanzapine reported by Citrome *et al*., 2009, (b) including only the data for 20 mg olanzapine reported by Citrome *et al*., 2009 and (c) including only the data for 40 mg olanzapine reported by Citrome *et al*., 2009

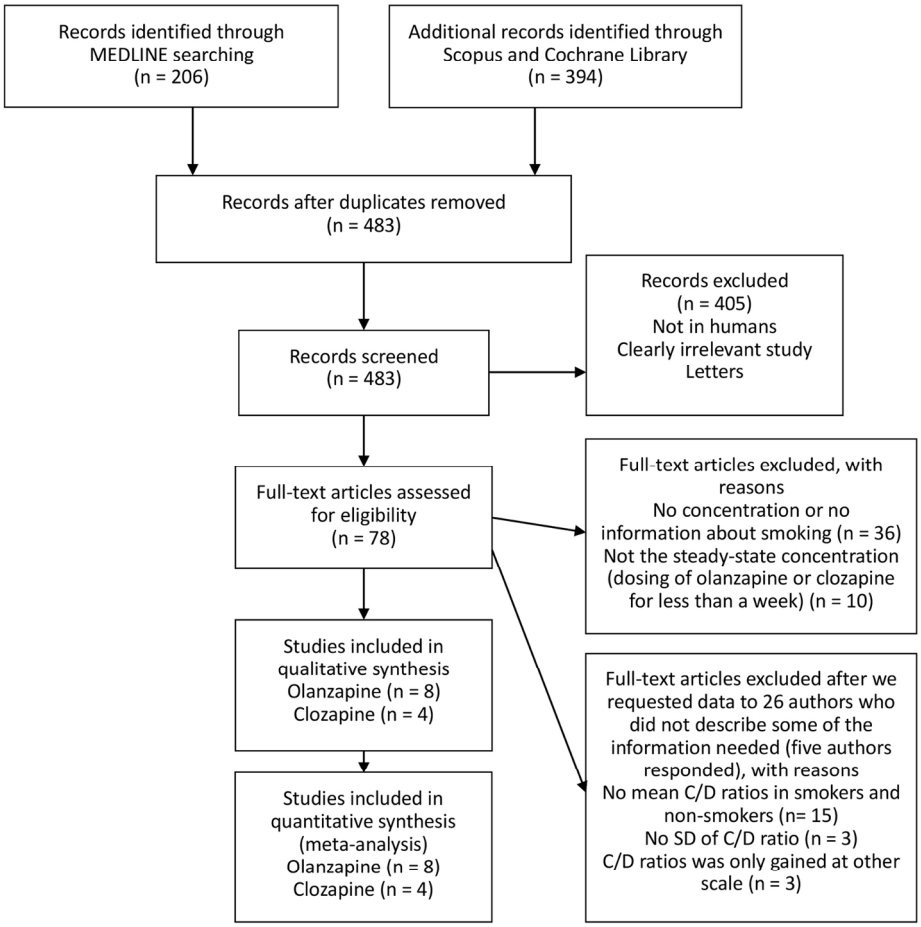

Figure 1
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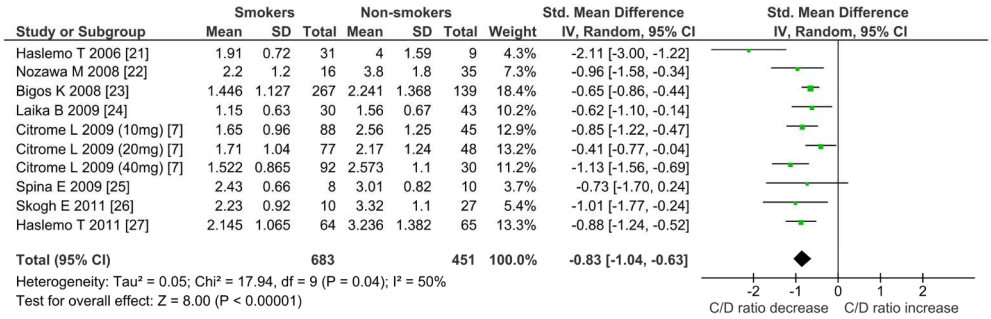

Figure 2
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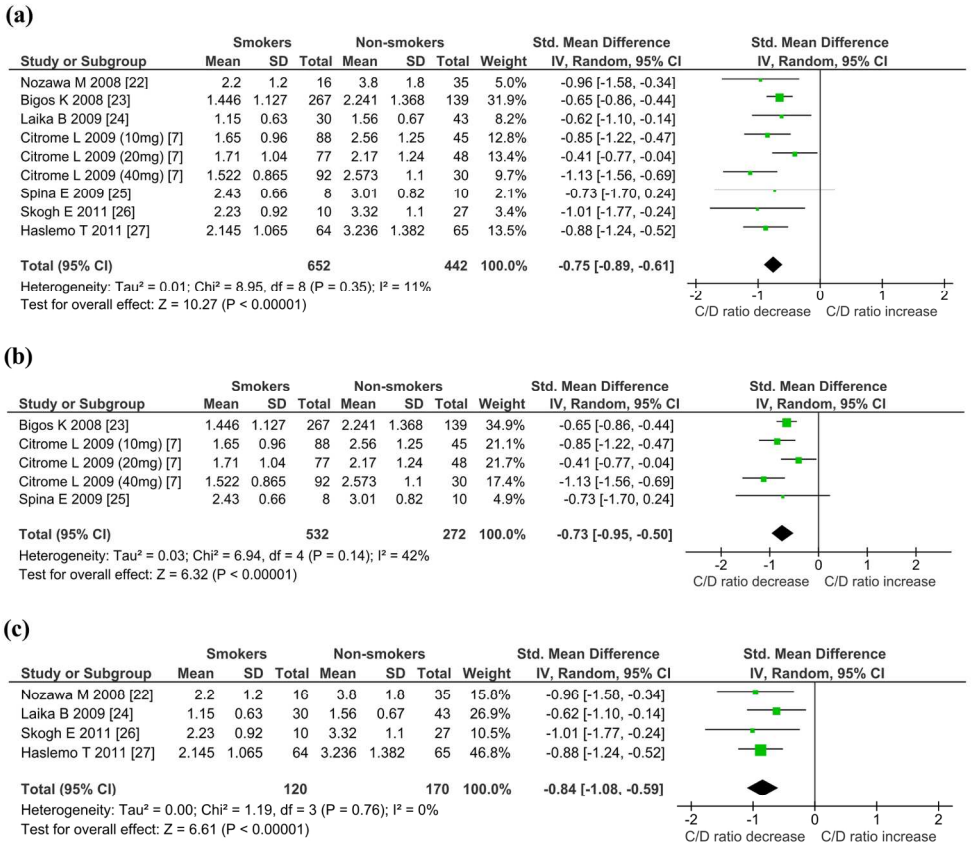

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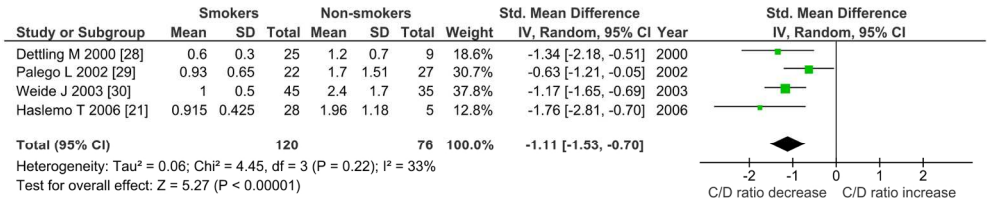

Figure 4
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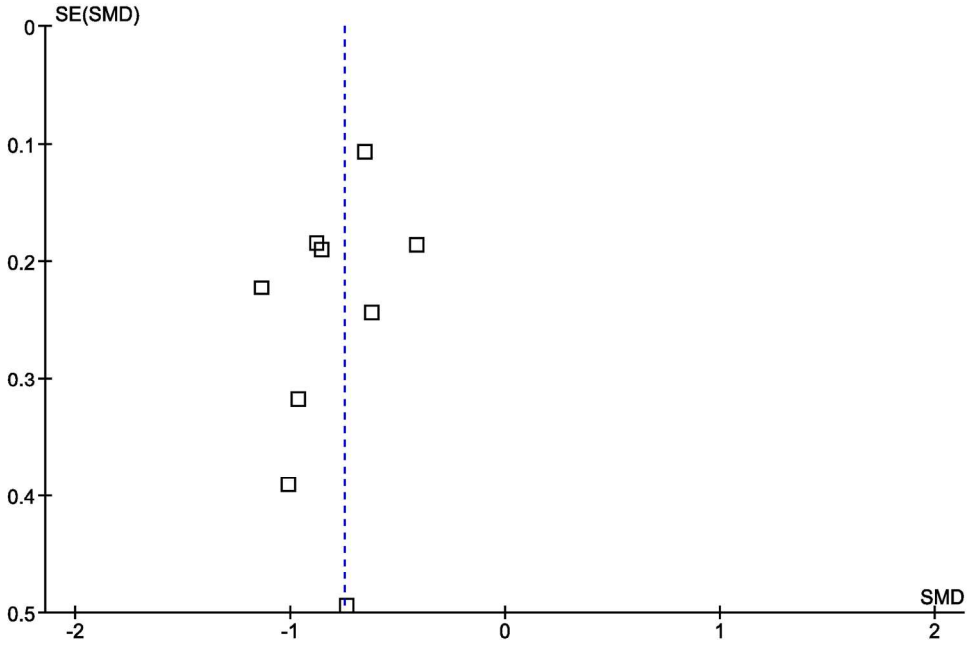

Supplementary Figure 1
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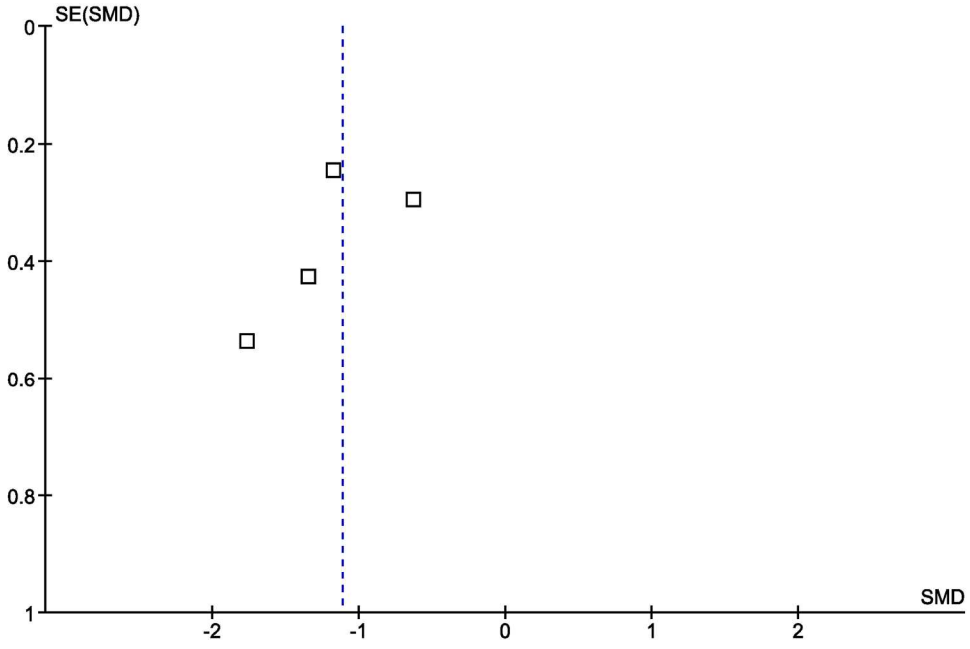
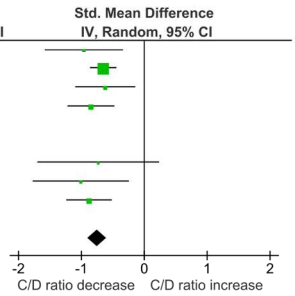


Supplementary Figure 2
170x113mm (300 x 300 DPI)

(a)

Study or Subgroup	Smokers Mean	SD	Total	Non-smokers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Nozawa M 2008 [22]	2.2	1.2	16	3.8	1.8	35	5.5%	-0.96 [-1.58, -0.34]
Bigos K 2008 [23]	1.446	1.127	267	2.241	1.368	139	48.1%	-0.65 [-0.86, -0.44]
Laika B 2009 [24]	1.15	0.63	30	1.56	0.67	43	9.3%	-0.62 [-1.10, -0.14]
Citrome L 2009 (10mg) [7]	1.65	0.96	88	2.56	1.25	45	15.2%	-0.85 [-1.22, -0.47]
Citrome L 2009 (20mg) [7]	1.71	1.04	77	2.17	1.24	48	0.0%	-0.41 [-0.77, -0.04]
Citrome L 2009 (40mg) [7]	1.522	0.865	92	2.573	1.1	30	0.0%	-1.13 [-1.56, -0.69]
Spina E 2009 [25]	2.43	0.66	8	3.01	0.82	10	2.3%	-0.73 [-1.70, 0.24]
Skogh E 2011 [26]	2.23	0.92	10	3.32	1.1	27	3.6%	-1.01 [-1.77, -0.24]
Haslemo T 2011 [27]	2.145	1.065	64	3.236	1.382	65	16.2%	-0.88 [-1.24, -0.52]
Total (95% CI)			**483**			**364**	**100.0%**	**-0.75 [-0.89, -0.60]**

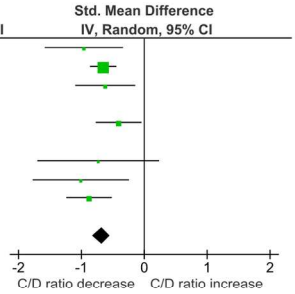
Heterogeneity: Tau² = 0.00; Chi² = 2.73, df = 6 (P = 0.84); I² = 0%
Test for overall effect: Z = 10.07 (P < 0.00001)



(b)

Study or Subgroup	Smokers Mean	SD	Total	Non-smokers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Nozawa M 2008 [22]	2.2	1.2	16	3.8	1.8	35	5.4%	-0.96 [-1.58, -0.34]
Bigos K 2008 [23]	1.446	1.127	267	2.241	1.368	139	47.7%	-0.65 [-0.86, -0.44]
Laika B 2009 [24]	1.15	0.63	30	1.56	0.67	43	9.2%	-0.62 [-1.10, -0.14]
Citrome L 2009 (10mg) [7]	1.65	0.96	88	2.56	1.25	45	0.0%	-0.85 [-1.22, -0.47]
Citrome L 2009 (20mg) [7]	1.71	1.04	77	2.17	1.24	48	15.8%	-0.41 [-0.77, -0.04]
Citrome L 2009 (40mg) [7]	1.522	0.865	92	2.573	1.1	30	0.0%	-1.13 [-1.56, -0.69]
Spina E 2009 [25]	2.43	0.66	8	3.01	0.82	10	2.2%	-0.73 [-1.70, 0.24]
Skogh E 2011 [26]	2.23	0.92	10	3.32	1.1	27	3.6%	-1.01 [-1.77, -0.24]
Haslemo T 2011 [27]	2.145	1.065	64	3.236	1.382	65	16.0%	-0.88 [-1.24, -0.52]
Total (95% CI)			**472**			**367**	**100.0%**	**-0.68 [-0.82, -0.53]**

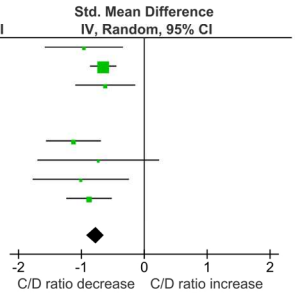
Heterogeneity: Tau² = 0.00; Chi² = 4.92, df = 6 (P = 0.55); I² = 0%
Test for overall effect: Z = 9.18 (P < 0.00001)



(c)

Study or Subgroup	Smokers Mean	SD	Total	Non-smokers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Nozawa M 2008 [22]	2.2	1.2	16	3.8	1.8	35	5.7%	-0.96 [-1.58, -0.34]
Bigos K 2008 [23]	1.446	1.127	267	2.241	1.368	139	50.1%	-0.65 [-0.86, -0.44]
Laika B 2009 [24]	1.15	0.63	30	1.56	0.67	43	9.7%	-0.62 [-1.10, -0.14]
Citrome L 2009 (10mg) [7]	1.65	0.96	88	2.56	1.25	45	0.0%	-0.85 [-1.22, -0.47]
Citrome L 2009 (20mg) [7]	1.71	1.04	77	2.17	1.24	48	0.0%	-0.41 [-0.77, -0.04]
Citrome L 2009 (40mg) [7]	1.522	0.865	92	2.573	1.1	30	11.6%	-1.13 [-1.56, -0.69]
Spina E 2009 [25]	2.43	0.66	8	3.01	0.82	10	2.4%	-0.73 [-1.70, 0.24]
Skogh E 2011 [26]	2.23	0.92	10	3.32	1.1	27	3.8%	-1.01 [-1.77, -0.24]
Haslemo T 2011 [27]	2.145	1.065	64	3.236	1.382	65	16.8%	-0.88 [-1.24, -0.52]
Total (95% CI)			**487**			**349**	**100.0%**	**-0.78 [-0.92, -0.63]**

Heterogeneity: Tau² = 0.00; Chi² = 5.21, df = 6 (P = 0.52); I² = 0%
Test for overall effect: Z = 10.23 (P < 0.00001)



Supplementary Figure 3
168x182mm (300 x 300 DPI)

MOOSE Checklist

Article details:

Title: Meta-analysis: The effects of smoking on the disposition of two commonly used antipsychotics, olanzapine and clozapine

Authors: Yoshiyuki Tsuda, Junji Saruwatari, Norio Yasui-Furukori

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Cigarette smoke increases the activity of CYP1A2, thus decreasing the blood concentrations of two commonly used antipsychotics, olanzapine and clozapine. However, no definitive agreement regarding the dose adjustment in clinical practice based on the patient's smoking status has been reached.
√	Hypothesis statement	It may be able to develop standards that can be used to adjust the doses of olanzapine and clozapine used in clinical practice based on the smoking status of the patient by conducting a meta-analysis.
√	Description of study outcomes	The mean concentration to dose (C/D) ratio (ng/ml)/(mg/day) of olanzapine and clozapine
√	Type of exposure or intervention used	Olanzapine or clozapine treatment
√	Type of study designs used	We included both prospective and retrospective studies.
√	Study population	The patients with schizophrenia or other psychiatric diseases who were treated with olanzapine or clozapine
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the investigators, Junji Saruwatari and Norio Yasui-Furukori are included in the author list.
√	Search strategy, including time period included in the synthesis and keywords	MEDLINE from 1946 – August 2012 Six terms in which either 'olanzapine' or 'clozapine' was paired with 'smoking' or 'cigarette' or 'tobacco' or 'smoke'.
√	Databases and registries searched	MEDLINE, Scopus and the Cochrane Library
√	Search software used, name and version, including special features	We did not employ any search software.
√	Use of hand searching	We hand-searched bibliographies of retrieved papers for additional references.

√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in Figure 1. The citation list is available upon request.
√	Method of addressing articles published in languages other than English	This meta-analysis excluded the article published in languages other than English.
√	Method of handling abstracts and unpublished studies	We did not search unpublished study.
√	Description of any contact with authors	We requested data from the authors if either the C/D ratio of olanzapine or clozapine or the standard deviation (SD) was not described.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria were described in the methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies provided mean C/D ratio and the SD values in smokers and non-smokers, respectively.
√	Assessment of confounding	We confirmed that race and sex could be associated with differences in the disposition of olanzapine using a meta-analysis. However, there was insufficient data available to assess the effects of these factors on the clozapine disposition.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	The quality of the included studies is shown in Table 3.
√	Assessment of heterogeneity	Heterogeneity of the studies was explored with I^2 statistics that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	The weighted mean difference of C/D ratios of olanzapine and clozapine between smokers and non-smokers was calculated by DerSimonian-Laird random effects models.
√	Provision of appropriate tables and graphics	Tables 1-3, Figures 1-4, and Supplementary figures 1-3
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figures 2-4
√	Table giving descriptive information for each study included	Tables 1 and 2
√	Results of sensitivity testing	We conducted subgroup analyses of olanzapine. The subgroup analyses (prospective/retrospective studies) also showed results similar to primary

		analyses of olanzapine. In the meta-analyses of clozapine, no subgroup analyses could be conducted because of the small number of patients included in the study.
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates.
Reporting of discussion should include		
√	Quantitative assessment of bias	Publication bias was not shown in both of analyses of olanzapine and clozapine using Egger test and funnel plot. In the present study, we excluded 10 reports (three about olanzapine and seven about clozapine) because the data were not from subjects who had received olanzapine or clozapine for at least a week (Figure 1). When the values were not described or they were given in another scale, we tried to gather information by requesting it from 26 authors, but only five authors responded to our requests. The other nine studies of olanzapine and 12 studies of clozapine could not be included (regarding olanzapine, the mean C/D ratios of olanzapine and its SD were not available for smokers and non-smokers in seven studies; the SD was not given in two studies. Regarding clozapine, the mean C/D ratios of clozapine and its SD were not available for smokers and non-smokers in seven studies; the mean C/D ratios were provided in another scale, i.e. (ng/ml)(mg/kg) in three studies and the SD was not given for two studies). Additionally, we excluded one study (i.e. Haslemo et al., 2006) in the analyses of olanzapine in order to reduce the heterogeneity. These may have led to a selection bias.
√	Justification for exclusion	We excluded the studies from subjects who have not received olanzapine or clozapine for at least a week.
√	Assessment of quality of included studies	We discussed quality of included studies in discussion section.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Based on the findings of the present study, it was estimated that if 10 and 20 mg/day of olanzapine (the usual doses in Japan) would be administered to smokers, about 7 and 14 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent olanzapine concentration. Based on the findings of the present study, it was estimated that if 200 and 400 mg/day of clozapine (the usual doses in Japan) would be administered to smokers, about 100 and 200 mg/day, respectively, should be administered to non-smokers in order to obtain the equivalent clozapine concentration.
√	Generalization of the conclusions	The findings of the present study suggest that the doses of olanzapine and clozapine should be reduced by 7/10 and

		1/2, respectively, in non-smokers compared with smokers in order to obtain an equivalent olanzapine or clozapine concentration. The results of this meta-analysis are useful as standards to regulate dosage of olanzapine and clozapine in clinical practice based on the patient's smoking status. However, this meta-analysis could not take the amount of smoking and adherence into consideration so additional research is required to establish administration plan based on smoking status.
√	Guidelines for future research	Future studies are required to investigate the effect of smoking on olanzapine and clozapine dispositions, while also taking the amount of smoking, adherence, and the other patient's characteristics (e.g., sex, race, genetic polymorphisms) into consideration.
√	Disclosure of funding source	This work was supported by grants-in-aid (Nos. 23510348, 24590652 and 25860117) for scientific research from the Japanese Ministry of Education, Science, Sports and Culture. Tobacco industry funding did not support the manuscript.

PRISMA flow chart: Figure 1